

Biology

Practical Guide



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Practical Work Guide

An Introduction

This guide is designed to provide material for supervisors to use at their discretion for the practical work.

Supplementary Practical Sheets cover those exercises which might be considered as optional.

All of the exercises provide opportunities to contribute to fulfilling the Aim that:

biology should encourage students to:

develop an understanding of scientific methods.

Candidates should be able to:

devise and plan experimental and investigative activities, selecting appropriate techniques;

demonstrate safe and skillful practical techniques;

make observation and measurements with appropriate precision and record these methodically;

interpret, explain, evaluate and communicate the results of their experimental and investigative activities clearly and logically using biological knowledge and understanding and using appropriate specialist vocabulary.

They provide the opportunity for the assessment of:

Planning

Implementing

Analysing evidence and drawing conclusions

Evaluating evidence and procedures

The responsibility for safe and correct practice rests entirely with the designated supervisor, to whom the methods are offered as a source of information only.

MICROSCOPY

The observation of animal and plant cells under the light microscope Introduction

A prepared slide of the mammalian small intestine in cross or transverse section (T.S.) is to be observed for the identification of the lining epithelium tissue.

The epithelial cells are column shaped, with an oval, darkly stained nucleus towards the bottom of the cell. The free edge of the cell has a 'brush' border of microvilli - but this will usually only be seen as a narrow 'different looking' layer at the top of the cell, where it faces into the lumen (space) of the gut. Goblet cell secreting mucus are also present, and quite distinctive.

Palisade mesophyll cells will be observed in a prepared slide of a leaf cut in cross section (T.S.). The palisade mesophyll cells form a layer (or layers) of cells below the upper epidermis of the leaf. They are oblong in shape, but may be quite badly disrupted in the preparation of the slide. Even under a high power (x40) objective lens it may be difficult to see cell structures, except for cell walls and chloroplasts.

Method

- ▼ Examine the prepared slide of T.S. leaf first. Locate the palisade cells using the medium power (x10) objective lens, you will probably need a high power (x40) objective lens to do the drawing.
- ▼ Draw 2-3 adjacent palisade cells (at least a few cm across), and label the structures you can identify. This may only be the cell walls, and the chloroplasts which are pushed up against them. Annotate the labels to indicate the functions of the parts you have drawn.
- ▼ Calculate the magnification of the drawing thus:

Magnification by microscope = Eyepiece lens magnification x objective lens magnification

Record this figure. Now correct the magnification for the size of the drawing, that is the number of times the drawing is larger than the image seen down the microscope, e.g. twice the size (x2).

This gives a final magnification of:

$\text{Eyepiece lens} \times \text{objective lens} \times 2$

Also record this figure

Examine the prepared slide of T.S. small intestine under the medium power (x10) objective lens.

The epithelial cells are much smaller than plant cells, and are much more indistinct as they lack the cellulose cell wall found in plant cells. You will need to move onto a high power (x40) objective lens once you have located the column shaped cells on the inner edge of the intestine wall. Draw 2-3 cells, enlarging them as much as you can. Label the structures you can identify and annotate the labels.

Calculate the final magnification of the drawing and record it. Make sure both drawings have a suitable heading.

- ▼ Now examine the prepared slide of the wall of the mammalian small intestine in cross section (T.S.) under medium ($\times 10$) objective lens. How many tissue layers can you identify in this specimen?
- ▼ Make a plan drawing of this specimen; do not show individual cells, just tissue layers. Label the different tissues and annotate your labels.
- ▼ Calculate the final magnification of your drawing
- ▼ Re-examine the prepared slide of T.S. leaf. Locate the palisade cells just under the upper epidermis using the medium power ($\times 10$) objective lens, and focus under the high power ($\times 40$) objective lens to do your drawing.
- ▼ Draw 2-3 adjacent palisade cells (at least a few cm across), and label the structures you can identify. These may only be the cell walls, and the chloroplasts which are pushed up against them. Annotate your labels.
- ▼ Calculate the final magnification of your drawing and record it.
- ▼ Re-examine the prepared slide of T.S. small intestine under the medium power ($\times 10$) objective lens. The epithelial cells are much smaller than plant cells, and are much more indistinct as they lack the cellulose cell wall found in plant cells. You will need to move onto a high power ($\times 40$) objective lens once you have located the column shaped cells on the inner edge of the intestine wall; with their 'brush' borders of microvilli. Mucous secreting goblet cells will also be visible.
- ▼ Draw 2-3 cells (not too small), label the structures you can identify, and annotate your labels.
- ▼ Calculate the final magnification of your drawing and record it.
- ▼ Make sure all drawings have suitable headings.

Teaching Notes

With plant tissues the student can usually see the cell walls and chloroplasts, the vacuole and nucleus. However, the cells can be quite badly distorted, poorly stained, and therefore difficult to draw. It might be a good idea for students to draw xylem elements in T.S. to gain some experience in drawing plant cells.

In the much smaller epithelial cells the nuclei should be seen, but they often overlap each other. The microvilli appear as a narrow layer along the free edge of the cells.

(It is interesting to note that many organelles were first described using the light microscope (some over 100 years ago) e.g. Golgi body (apparatus), mitochondria, and endoplasmic reticulum).

This practical also provides an opportunity to discuss the main points of **biological drawing technique**.

The important points to get across are:

- ▼ Drawings should be line drawings, with no shading, or use of colour.
- ▼ They should be large enough to be clear.
- ▼ The lines should not be sketchy.
- ▼ Pencils should be sharp, HB or H.
- ▼ Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.
- ▼ Labels should be annotated, either next to the label or as a suitable note, to give further information as required.
- ▼ Magnification, or an indication of size/scale, should be included.
- ▼ Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section / squash etc., and preparation or staining details.
- ▼ Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

Requirements per student

Microscope

Drawing materials

Prepared slides of T.S. leaf

Prepared slides of T.S. mammalian small intestine

The preparation of temporary mounts, the use of simple staining techniques

Introduction

Plant and animal cells are to be studied under the light microscope. The plant cells are to be viewed by making a temporary preparation of the inner epidermis of an onion scale, stained with iodine in potassium iodide solution to make the cells more visible. A prepared slide of human epithelial cells from the lining of the cheek will also be studied.

Method

- ▼ Place 1-2 drops of iodine in KI solution in the middle of the slide.
- ▼ Take a piece of onion scale and tear it with the fingers. This should leave a very thin epidermis attached to the inside surface of the scale. Carefully remove a small piece (about 0.5 cm²) of this epidermis with the forceps, and place it in the drop of iodine solution on the slide.
- ▼ Use the mounted needle to position the piece of epidermis flat in the iodine solution, then use the needle again to lower the coverslip carefully onto the specimen, (trying not to trap too many air bubbles).
- ▼ Examine the slide under the light microscope, using the medium power (x10) objective lens, and draw 2-3 adjacent cells (at least a few cm across). Label the drawing to show the structures you can see, e.g. nucleus, cell wall, vacuole, and cytoplasm (they may not all be visible). Annotate the drawing to show the functions of the structures you have drawn.
- ▼ Calculate the magnification of the drawing:
$$\text{Microscope magnification} = \text{eyepiece lens magnification} \times \text{objective lens magnification}$$

Note this figure on your drawing.
- ▼ Now correct the magnification for the size of the drawing; that is the number of times the drawing is larger than the image seen down the microscope eg, twice the size [x2]. This gives a final magnification of:
$$\text{Eyepiece lens} \times \text{objective lens} \times 2$$

Also note this figure on your drawing.
- ▼ Use the high power (x40) objective lens to check the details you have drawn.
- ▼ Now examine the prepared slide of human cheek cells under the microscope. Locate the cells using the medium power (x10) objective lens, you will probably need a higher power to make the drawing.
- ▼ Draw 2-3 cells (not too small), label the drawing, annotate, and note the final magnification (microscope magnification x size difference).
- ▼ Make sure the drawings have suitable headings.

Teaching Notes

This practical usually works well, provided the student gets a clean piece of the onion epidermis, without any additional tissue. They also need to make sure it is flat under the coverslip, and hopefully without too many air bubbles.

The student can usually see the vacuole and cell wall clearly. They may see the nucleus, which is usually pushed over to one side of the cell by the vacuole. Occasionally it is possible to see the cell membrane in the corners, if the cells have plasmolysed slightly. Cytoplasm is usually visible as a granular material in the corners of the cells.

The cheek cells have less detail, although the nucleus can be clearly seen. Some cells may show granules in the cytoplasm.

This practical also provides an opportunity to discuss the main points of **biological drawing technique**. The important points to get across are:

Drawings should be line drawings, with no shading, or use of colour.

They should be large enough to be clear.

The lines should not be sketchy.

Pencils should be sharp, HB or H.

Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.

Labels should be annotated, either next to the label or as a suitable note, to give further information as required.

Magnification, or an indication of size/scale, should be included.

Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section/squash etc, and preparation or staining details.

Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

Requirements per student

- Microscopes
- Slide and coverslip
- Forceps
- Mounted needle
- Drawing materials
- Iodine in potassium iodide solution
- Pieces of fresh onion scale
- Prepared slide of Human cheek cells

To investigate the movement of water down water potential gradients through partially permeable membranes

Introduction

The changes in mass of tissue caused by water loss or gain, due to osmosis at the cellular level, are to be investigated. Pieces of plant food storage tissue, e.g. potato, will be placed in distilled water, and sucrose solutions of different concentrations.

Method

- ▼ Collect a set of six 50cm³ beakers, and label them 0%, 0.5%, 1%, 5%, 10%, and 15%. Put 30 cm³ of distilled water in the 0% beaker.
- ▼ Using the 15% stock solution of sucrose supplied, make up the dilutions of sucrose solution listed, and put 30cm³ of each dilution in the appropriate labelled beaker.
- ▼ Collect a potato and use the cork borer to cut out a cylinder of potato tissue. Carefully cut the cylinder into discs 1mm thick, discarding the ends with the skin on. You will need about 60 discs.
- ▼ Using a balance, weigh 10 of the potato discs, note the mass and record in a suitable table. Repeat 5 times, so you have 10 discs of known combined mass for each solution.
- ▼ Add 10 discs of known combined mass to each of the solutions. Note the time.
- ▼ After 45-60 minutes, remove each set of discs from the beakers, very carefully blot them dry on a paper towel or tissue and reweigh them. Make a note of the new masses in the table.
- ▼ Calculate the % change in mass of the potato tissue in each liquid by dividing the gain or loss in mass by the original mass and multiplying by 100. Plot a graph of % change in mass against concentration of sucrose solution, include the distilled water result.

Discuss what the results indicate about osmosis and the effect that it has on living tissue.

Use the graph to estimate which concentration of sucrose solution possibly matches the internal concentration of the potato cells.

Teaching Notes

This practical works well, and is better than the standard potato chip exercise, as the surface area / volume ratio is higher so the uptake / loss of water gives clearer results. The discs are fairly easy to cut, but to save time, the discs can be cut beforehand and kept in 5% sucrose solution.

Some students may need help calculating the volumes of sucrose solution and distilled water needed to obtain the correct dilutions.

If possible you need a balance which will show 2 decimal places to get an accurate result.

Students must be very gentle when blotting discs before reweighing

(Changes in onion ring diameter in different concentrations of solution/changes in mass of slices of banana of different ripeness/comparing change in the mass of slices of different plant materials e.g. carrot, parsnip, swede etc. are all worth a try).

Requirements per student

Cutting tile
1.5 – 2 cm diameter cork borer
Sharp knife
6 x 50 cm³ beakers
50 cm³ measuring cylinder
Syringes/pipettes
Balance
Paper towels/tissues
100cm³ 15% sucrose solution
Distilled water
Potato

The observation of arteries, veins and capillaries under the light microscope

Introduction

The observation of these vessels under the light microscope is typically of cross or transverse sections (T.S.), although capillaries are more likely to be cut in other ways in a cross section of the tissue in which they are found. The appearance of the detail will vary with the staining technique.

The artery and vein are usually straightforward to identify, although sometimes the vein is mistaken for a large space in the tissue. It may, however, be difficult to distinguish between the various tissues in the vessel wall. Capillaries are difficult to identify with any certainty in cross section at this level. Perhaps the best way is to observe a T.S. mammalian lung, identify an alveolus, then look for the capillaries that surround it. The nuclei of the endothelial cells and the sectioned red blood cells may also help in identification.

Method

- ▼ Collect a prepared slide showing a section through a mammalian artery and vein. Examine this under the microscope, using the magnification you find most suitable.
- ▼ Draw a plan of each vessel, showing the layers of tissues only (no details of cells), and label your drawing.
- ▼ Record the final magnification (eyepiece $\times$ objective $\times$ size difference) near the title of your drawing.
- ▼ Now examine a prepared slide of a section of mammalian lung tissue, and try to identify the alveoli and capillaries next to them using the high power ($\times 40$) objective lens. The capillaries are lined with an endothelium of squamous cells, but you will be probably only be able to see their stained nuclei. The capillaries may contain red blood cells, cut in section.
- ▼ If possible make a drawing of the capillary; enlarge it as much as you can.
- ▼ Label your drawing and record its final magnification. Do not forget a heading.
- ▼ Draw and complete a suitable table to compare the differences and similarities in structure and function between the three types of blood vessel.

Teaching Notes

The artery and vein are usually straightforward, provided the slide is clear, although sometimes the vein is mistaken for a large space in the tissue.

The capillary is harder to identify, because of its size. The best way is to identify an alveolus, then look for the capillaries that surround it. The nuclei of the endothelial cells and the sectioned red blood cells may also help

Details of the relative sizes are important to show the variation in size of these three types of vessel.

Measurement of the artery, vein and capillary would give a good opportunity to use the eyepiece graticule.

Requirements per student

Microscope

Prepared slide of a T.S. mammalian artery and vein

Prepared slide of a T.S. mammalian lung tissue

Drawing materials

Reference books/Photographs and/or diagrams of appropriate tissues

The microscopic examination of stained blood films and the identification of cells

Introduction

Red blood cells are one of the smallest cells you will view under the light microscope, and although the smear is large enough, care still has to be taken to find the focus with the high power ($\times 40$) objective lens. The nuclei of white blood cells stain dark blue and when present in the smear are easily seen, however, they are few and far between and you may need to look at a number of fields of view to see examples of the different types of white cell. They are usually easier to spot using the medium power ($\times 10$) objective lens, you can then move to higher power to draw the cells.

Method

- ▼ You are provided with a prepared slide of a human blood smear. Examine it under the microscope using medium power ($\times 10$) objective lens, and then high power ($\times 40$) objective lens.
- ▼ Identify the various types of blood cell: red cells, granulocytes, lymphocytes, and monocytes.

Granulocytes 72% (Lobed nucleus and granular cytoplasm)
Lymphocytes 24% (Large circular nucleus fills most of the cell).
Monocytes 4% (Larger cell with large kidney shaped nucleus).
- ▼ Draw examples of each type of cell, label them and annotate your drawings with details of their functions.
- ▼ Record the final magnification of your drawings (eyepiece $\times$ objective $\times$ size difference) near to the heading.
- ▼ Platelets are usually visible as clusters of small purple 'granules' in the plasma.

Teaching Notes

The nuclei of the leucocytes are stained purple; the cytoplasm is usually pale, with purple or pink-stained granules.

The different types of granulocyte, lymphocyte, and monocyte are found in the following proportions (as % total white cell count)

Granulocytes	72%	(70% neutrophils, 1.5% eosinophils, 0.5% basophils)
Lymphocytes	24%	
Monocytes	4%	

Requirements per student

Microscope

Prepared slide of Human blood smear

Drawing materials

Reference books/photos etc.

Practical Work Sheet 'Measuring specimens under the microscope'

Microscopic examination of vertebrate tissues - the ovary and testis

Introduction

The section of the testis should be clear, but remember, in cross section, the single coiled seminiferous tubule appears as a collection of apparently unconnected circles.

The section of the ovary represents a moment in time in the ovarian cycle. Each follicle develops and matures in one position in the ovary, and at any one time several follicles will be caught at various stages of development. A range of slides may have to be examined before all stages are seen. Remember that theoretical diagrams typically show all stage of development of the follicles around the ovary in sequence, even giving the impression that one follicle travels around the ovary during its development.

Keep these points on the interpretation of sections as seen under the microscope in mind, as you examine the slides.

Method

- ▼ Collect a prepared slide of a transverse section (T.S.) through the mammalian testis.
- ▼ Examine the slide under the microscope, first using the (x4 or x10) objective lenses, and identify the seminiferous tubules.
- ▼ Next examine a single seminiferous tubule under high power (x40) objective lens, and try to identify the spermatogonia, primary spermatocytes, spermatids, Sertoli cells and developing sperm. The nuclei of cells are clearly seen, but it is often difficult to make out cell boundaries.
- ▼ Using the medium power (x10) objective lens draw a plan of the seminiferous tubules, and using the high power (x40) objective lens make a detailed drawing of part of the wall of one tubule. Compare what you see with photographs and diagrams, and label and annotate your drawings.
- ▼ Now examine the prepared slide of the longitudinal section (L.S.) of the ovary, using the medium power (x10) objective lens. Identify any developing follicles that are visible. Make a simple low power drawing of what you see. Compare what you see with photographs and diagrams, and label and annotate your drawings.
- ▼ Make sure your drawings have suitable headings.

Teaching Notes

If the mammal from which the slides have been made is identified, try to find out about the pattern of oestrus shown by that animal, as this will prevent the students assuming that there is a monthly cycle in the ovary in question.

Requirements per student

Microscope
Prepared slides of T.S. testis
Prepared slides of L.S. mammalian ovary (often rabbit)
Drawing materials
Reference material

Measuring and Counting Cells

Introduction

There are two methods you can use to measure the size of objects under the light microscope. One is a calculation based on the magnification, the other is to use an eyepiece graticule, which will need to be calibrated for the microscope using a stage micrometer. The graticule will give you a more accurate measurement, but the procedure is more complex.

Method A - Using the magnification

Make a note of the magnification of the eyepiece lens, and of the objective lens. The total magnification is found thus:

Total magnification = magnification of eyepiece lens $\times$ magnification of objective lens

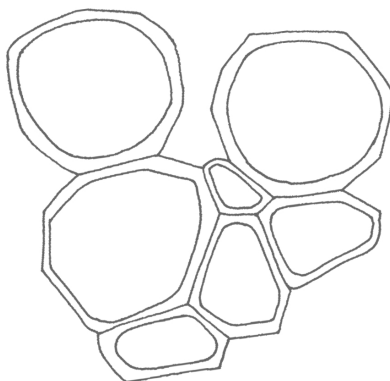
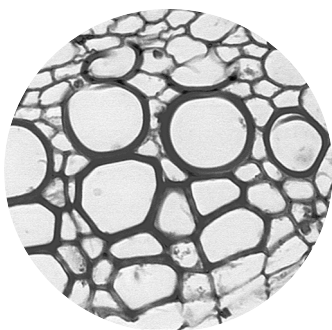
The term **linear magnification** refers to the number of times an imaginary 'line' across the drawing is greater than the equivalent would be on the original specimen (as opposed, for example, to considering the magnification of the area).

To calculate the linear magnification of the drawing, estimate how large the drawing is, compared with the view down the microscope. Look down the microscope, and then at the drawing, is the drawing $\times 2$, $\times 3$, $\times 4$ the size of the specimen?

You can then multiply the magnification by the size difference.

So if this is what you can see:
(with $\times 100$ magnification)

and this is what you have drawn:



If the eyepiece lens is $\times 10$, and the objective lens is $\times 10$, then:

Final magnification = $10 \times 10 \times 2$ (drawing is about twice the size of the specimen) = $\times 200$ magnification

To work out the size of the original object under the microscope, measure a line across the drawing in mm, and divide it by the total magnification, giving the answer in μm .

Teaching Notes

This practical provides an opportunity to discuss the main points of **biological drawing technique**. The important points to get across are:

Drawings should be line drawings, with no shading, or use of colour.

They should be large enough to be clear.

The lines should not be sketchy.

Pencils should be sharp, HB or H.

Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.

Labels should be annotated, either next to the label or as a suitable note, to give further information as required.

Magnification, or an indication of size/scale, should be included.

Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section/squash etc, and preparation or staining details.

Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

In this exercise students must be reminded not to put too much detail in their drawings, only layers or regions of tissues are required, no details of cells.

Some may be interested in checking their linear magnification estimation by observation of a structure of consistent, known size, e.g. the diameter of red blood cells (6-8 μm).

Requirements per student

Microscope

Drawing materials

Reference books / pictures / class notes on plant and animal histology

Prepared slide of leaf TS

Supplementary Practical Sheet: Measuring specimens under the light microscope

Eye piece graticule. e.g. in acetate strip

Slide micrometer

Measuring the size of microscopic structures

Method B - Using the eye piece graticule

- ▼ To do this, you need to have a scale (graticule) in position in your eyepiece, so that it can be seen when you look down the microscope. These scales are usually on small circular pieces of glass or acetate.
- ▼ To insert the scale in your eyepiece, remove the eyepiece lens from the microscope, and carefully unscrew the top lens. If you look down into the lens body, you should see a ledge running round the sides about halfway down. Drop the scale into the lens body, so that it rests on the ledge, then replace the top lens. It does not matter if the scale is the wrong way up, but if it annoys you, unscrew the lens again and turn the scale over.
- ▼ When you look through the microscope, you should see the scale overlying your specimen.
- ▼ To calibrate the scale, you need to use a stage micrometer. This can be a special slide with a scale engraved on it, or one can be made by mounting a scale on a slide under a coverslip using Canada Balsam. It usually consists of a scale 1cm long, which is divided into 100 units, each of which is 0.1 mm (100 μm), there is an extended line every 10th unit. It is worth being aware that these scales can be badly scratched.

continued

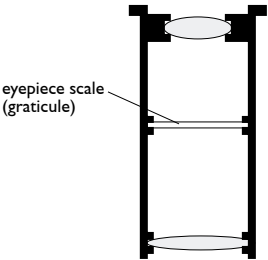
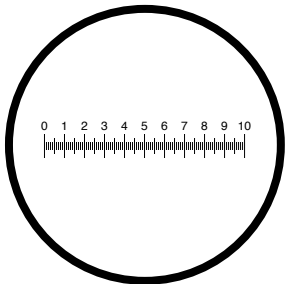
To calibrate the eyepiece scale

- ▼ Place the stage micrometer on the microscope stage and hold it down with the clips.
- ▼ Using the eyepiece lens with the scale in, look through the microscope and focus it so you can see both scales clearly. This is usually easier if you focus your eye on the eyepiece scale and adjust the microscope so the stage scale comes into focus as well.
- ▼ Move the stage micrometer carefully so that the starting units of the two scales coincide.
- ▼ Count along the two scales until there is again a coincidence between the two scales. Note down the number of divisions along each of the two scales that this represents.
- ▼ As each division along the stage micrometer scale represents 100 µm, then:
$$1 \text{ division on the eyepiece scale (in mm)} = \frac{\text{Number of divisions on the stage micrometer scale}}{\text{Number of divisions on the eyepiece graticule scale}} \times 100$$
- ▼ At x100 and x400 magnification the lines on the scale will have a definite thickness. It is important to measure from one side of one scale mark, to the same side of the next coinciding mark.
- ▼ Repeat this procedure for all the objective lenses on your microscope, so that you have a scale calibration for all magnifications. Record this information in a table with the following headings.

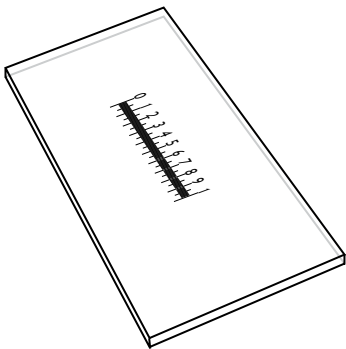
Power of lens	Magnification	Eyepiece scale 1 division (in mm)

- ▼ When you measure another specimen, you will already have the calibration figures, so all you have to do is count how many eyepiece scale divisions your specimen covers, and multiply that by the calibration factor for that objective lens.

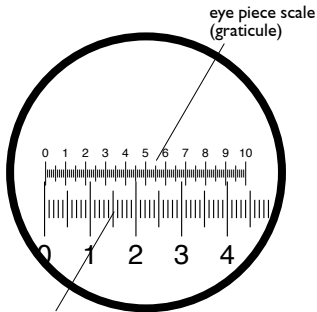
Eyepiece scale (graticule)



Eyepiece scale as seen when held up to the light away from the microscope.

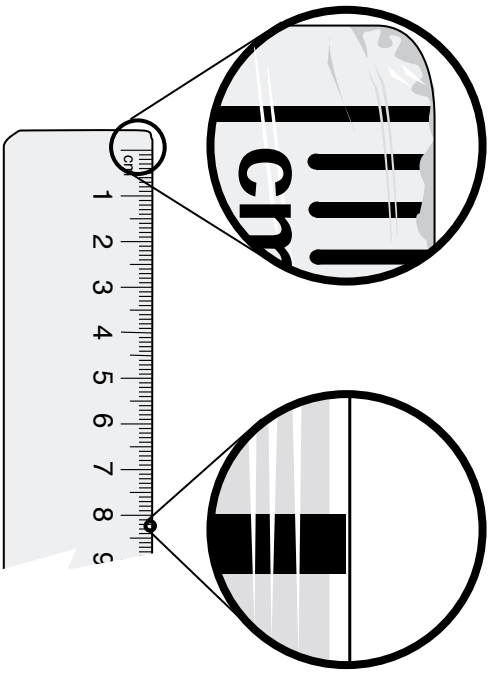


Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

More of the object is seen under low power objective lens



Less of the object is seen under high power objective lens

Teaching Notes

Measuring the Size of Cells

Requirements per student

- Microscope
- Eyepiece graticule
- Slide micrometer
- Suitable prepared slides

Reference books / pictures / class notes on plant and animal histology

Practical notes

Individual help is often required to ensure that students fully understand the procedure of using the eye piece graticule.

The plastic ruler analogy may help, and by giving an estimate of the diameter of the field of view can actually provide a crude measure of size of microscopic structures seen under the light microscope.

Counting Cells using a haemocytometer

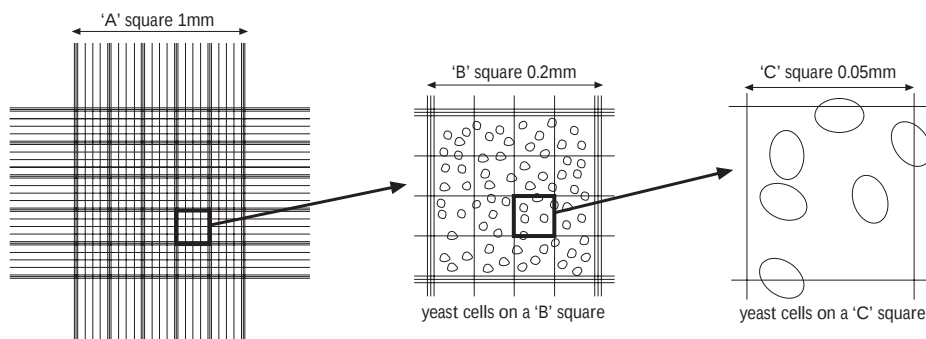
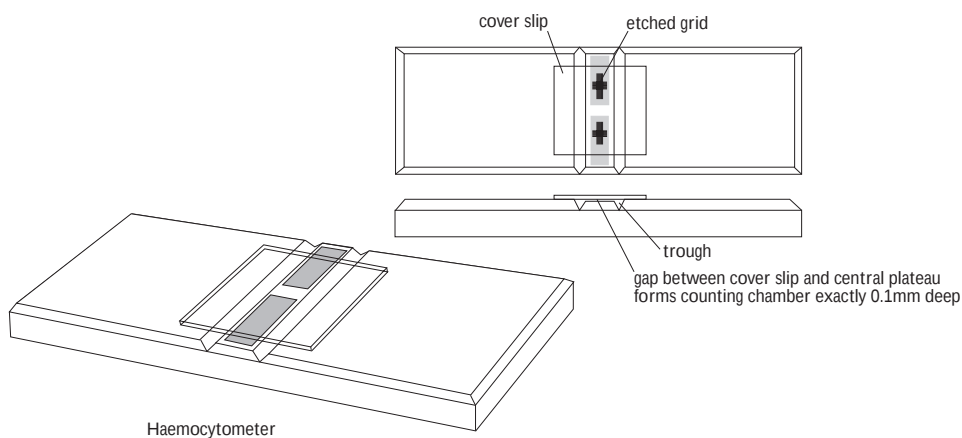
Introduction

A haemocytometer, was originally devised to count blood cells. It has a grid marked on it, on which rests a known volume of liquid. By counting the number of cells visible against the grid, multiplying by the known volume, an estimate for cell numbers can be obtained. (An advantage of this method if using bacteria is that results are obtained quickly, rather than having to inoculate agar plates and incubate them before counting colonies).

Method

- ▼ Collect a sterile 250cm³ conical flask containing malt extract broth, together with a test tube containing an active yeast culture.
- ▼ Using aseptic technique as demonstrated by the supervisor, add 1cm³ of yeast culture to the conical flask and swirl it carefully to mix. Put some cotton wool in the neck of the flask.
- ▼ Examine the haemocytometer slide by eye. There are usually two grids. You should just be able to see the markings in the centre of the two middle sections of the slide, either side of a central channel. These form grids of 1 mm², divided into smaller squares, as shown in the diagram. The central section of the slide is slightly lower and gives a precise depth when the coverslip is in position.

- ▼ Place a coverslip over the haemocytometer grids, pressing down firmly but carefully. If the coverslip is in the correct position, you should be able to see coloured rings under the coverslip, like the rings you see on a soap bubble: they are called “Newton’s Rings”. Using a sterile dropping pipette and aseptic technique, remove a small sample of the Yeast culture and run a drop under the coverslip of the haemocytometer (any excess solution runs off into the side channels).
- ▼ Examine the haemocytometer under the microscope. Adjust the microscope until you can see the yeast cells overlying the grid of the slide.
- ▼ Decide whether you are going to count cells which are budding as one cell or two. You also need to decide how you deal with cells that lie over the gridlines. The usual method is to count cells overlapping two sides of the grid, but not those overlapping the other two sides. (See diagram). Once you have decided these two points, stick to them.



continued

- ▼ Count the number of yeast cells in a selection of squares in the grid. To get an accurate estimate, you need to count about 150-200 yeast cells. Make a note of the number of squares you count, and the number of yeast cells per square to give a total. Use these figures to estimate the number of yeast cells per cm^3 of the culture. The volume of the whole grid is 0.1mm^3 , of a large square is 0.004mm^3 , and of a small square is 0.00025mm^3 . Use these figures as appropriate to calculate the number of yeast cells in the culture.
- ▼ Leave the yeast culture to incubate at $20\text{-}25^\circ\text{C}$ for 4-7 days. Count the number of cells, using the haemocytometer, at least once per day, making a note of the exact time when the culture was sampled. Remember to swirl the flask before sampling, to mix the cells evenly.
- ▼ Plot the results on ordinary graph paper, as cell numbers against time. You also need to make a plot of Log_{10} cell numbers against time. You can do this either by calculating the log_{10} figures from the data, or by using 'semi-log' graph paper, which has the lines already marked as a logarithmic scale.
- ▼ Compare the two graphs in your account

Teaching Notes

Requirements per student/group:

Haemocytometer Sterile

1 cm^3 pipette

Pipette filler

Several sterile dropping pipettes

Sterile 250 cm^3 conical flask, containing 50 cm^3 sterile malt extract broth

Active yeast culture

Preparation of an onion root tip squash to observe stages in mitosis

Introduction

A preparation of a slide of an onion root tip shows the stages of mitosis in the dividing cells. The tip must be softened so the cells can be squashed out and examined, it is also necessary to stain the chromosomes to see the stages of mitosis.

Method

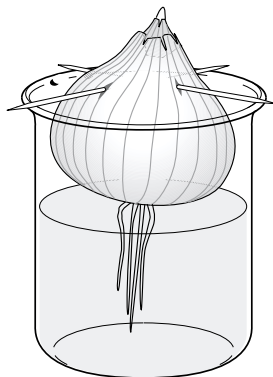
- ▼ Remove a root from a growing onion bulb. Carefully cut about 0.5cm from the tip of the root, making sure it is the tip end, not from the end near the onion. (It should be slightly pointed)
- ▼ Place the root tip in a watch glass. Add 9 drops of acetic orcein stain, and 1 drop of molar hydrochloric acid. Heat the watch glass gently, using a hotplate or spirit burner, or by passing it carefully but quickly through the flame of a bunsen burner: DO NOT LET IT BOIL! Keep the watch glass hot but not boiling for 5 minutes: if you have to hold it, use forceps.
- ▼ Place the root tip on a clean slide, and add a drop of 45% ethanoic acid. Cut away all but the terminal 1mm. Cover with a clean coverslip.
- ▼ Squash the root tip gently using a pencil: hold the pencil vertically just above the coverslip, then let it fall through your fingers onto the coverslip. Repeat this 2-3 times. This should spread the cells out under the coverslip so they can be examined under the microscope.
- ▼ Examine the slide under the microscope, using medium power (x10) objective lens and then high power (x40) objective lens. Identify the various stages of mitosis, and make annotated drawings of each stage.
- ▼ Record the final magnification of your drawing (eyepiece x objective x size difference).

Teaching Notes

Growing the onion roots needs to be started 6-7 days before the practical. It is best to use onion sets from a garden centre, or locally grown onions, as some onions bought from a supermarket have been treated to prevent sprouting.

Push two cocktail sticks through the onion at right angles: these will support the onion in the beaker, as shown in the diagram. Fill the beaker with water until it just covers the root area of the onion, and leave it in a cool place until the roots have sprouted. They do not have to be kept in the dark. Ideally, the roots need to be about 2 - 5 cm long.

If the onions are ready before the lesson is due, or if you wish to harvest onion roots and store them until needed, they can be treated with Carnoy's solution (6 parts absolute ethanol: 3 parts chloroform: 1 part glacial acetic acid) for 24 hours, then stored in 70% ethanol until needed. They should be removed from the Carnoy's solution before being given to the students if the lesson is within 24 hours of harvesting.



The watch glasses need to be heated gently for five minutes, but they should not be allowed to boil or dry out. If they begin to dry out, an extra drop of stain can be added.

If the students have trouble finding mitotic cells in their specimen, check that they have actually used the root tip: if you can see developing xylem or phloem, they have retained the wrong portion.

It can be useful to have a few prepared slides of root tip squashes or sections, in case the students have difficulty with the slide preparation. Appropriate photographs and / or diagrams will also be useful. In addition, it will be helpful if the students can see video footage of mitosis. It is important to make it clear that mitosis is a continuous process, which is subdivided for convenience.

It might be possible for the students to estimate the relative length of each stage of mitosis, by counting how many cells they can see which are in each stage.

Requirements per student

Watch glass
Forceps, scalpel/knife
Slide and coverslip
Bunsen burner/spirit burner
Microscope

Requirements for class

Growing onions: see previously
Acetic orcein stain in dropping bottles
1 molar hydrochloric acid in dropping bottles
45% ethanoic acid, in dropping bottles
Adjustable hotplate, if available
Beaker of detergent for the used slides
Book illustrations, photos etc.

Experimental investigation into the factors affecting the growth of pollen grains

Introduction

The growth of pollen tubes which are normally formed as the pollen grains germinate on the stigma of the flower, can be induced by keeping them in sucrose solution. Various factors that affect the growth of the pollen tube can be investigated.

Method

- ▼ Collect a small quantity of pollen grains from one of the plants provided. Using a paint brush, transfer a few grains to a slide and add a drop of water and a cover slip. View the grains under a microscope with the medium power (x 10) objective lens. Note the distinctive pattern on the surface of the pollen grains. Each type of plant has a characteristic pattern.
- ▼ Transfer another sample of pollen grains into the well of a cavity slide. Add 2-3 drops of sucrose solution and stir gently with a cocktail stick to ensure the pollen grains are wetted by the solution.
- ▼ Put your slide in an incubator at 25°C, and leave the pollen grains to germinate. Remember to top up the sucrose solution (with sucrose solution at 25°C) if the slides start to dry up.
- ▼ Examine the slide at 15 minute intervals. It may be quite a long time before the pollen tubes appear, and you may be provided with pre-prepared samples if your time is short.
- ▼ Once the pollen tubes have formed put a coverslip on and irrigate the slide with acetocarmine or neutral red to stain the nuclei (pollen tube nucleus and male gametic nuclei).
- ▼ Continue to incubate and observe every 15 minutes, note the time at which the pollen tubes are at least as long as the width of the pollen grain.
- ▼ Working in a small group, identify factors that you think might affect the growth of pollen tubes, either positively or negatively. Devise investigations to test your predictions. Decide how you are going to measure the growth of the pollen tubes.
- ▼ Check your plans with your supervisor, then carry out your investigations.
- ▼ Report on your findings.

Teaching Notes

This practical should provide a good potential extension exercise for your more able students, who might try some of the more complex factors, such as the presence of stigma extract.

Most flowers will provide some pollen, so use whatever may be available at the time of year. If you are doing this practical during the winter, flowers can be bought from a florist or supermarket. Among the varieties we have tried Lilies work best (other authors recommend *Tradescantia* and *Nasturtium*).

In the summer wind borne pollen grains can be collected on slides coated with glycerine, or sticky tape left in a position exposed to air currents, e.g. a window ledge.

Suggested factors to test are:

- temperature;
- light levels;
- pH;
- different concentrations of sucrose;
- extract of stigma tissue (prepared by chopping up stigmas removed from the flowers in a few drops of sucrose solution): this could be from the same flower or different ones;
- presence of different pollens;
- presence of plant hormones.

Requirements per student

- Microscopes
- Slides and coverslips
- Cavity slides and coverslips
- Cocktail stick
- Paint brushes
- Incubator or equivalent
- Calibrated eye piece graticules
- 0.4% sucrose solution, a few cm³ (some recommend as high as 3%)
- Acetocarmine stain or neutral red
- Plant material, with flowers producing pollen.

Tests for substances of biological importance

Introduction

A standard series of procedures are used to test for a variety of biological molecules. These tests are traditionally known as 'food tests', but here the basic test methods are performed on a standard solution of each type of substance.

Method - Benedict's test for reducing sugars

- ▼ Put on a pair of safety glasses. Collect a 500cm³ beaker and fill it about half full with tap water. Place it on a tripod and gauze over a Bunsen burner and bring the water to the boil.
- ▼ Place a small quantity of reducing sugar solution (about 1-2 cm³) in a boiling tube. Add 2 cm³ of Benedict's reagent.
- ▼ Place the boiling tube in the waterbath and boil the tube and its contents for 3-4 minutes. Remove the tube from the waterbath using test-tube tongs and place it in a test-tube rack.

If the sample contains reducing sugars, a precipitate will be produced, and the colour will change from blue through green and yellow to brick-red, depending on the amount of sugar present. This test can thus be used to give an estimation of the amount of sugar present, provided the quantities of sugar solution and Benedict's reagent used are the same each time. A small amount of sugar will turn the solution green, larger amounts yellow and considerable quantities will turn it brick-red.

continued

Method - Benedict's test for non-reducing sugars

This test should be done if the previous test for reducing sugars gives a negative result.

- ▼ Place a fresh sample of sugar solution (1-2cm³) in a boiling tube and add 1cm³ dilute hydrochloric acid. Place the boiling tube in the waterbath and boil it for 1-2 minutes.
- ▼ Remove the test-tube from the waterbath using tongs and place it in a test-tube rack. Allow to cool for a couple of minutes. Using a spatula, add small quantities of sodium hydrogen carbonate powder, until the mixture stops fizzing. (This will probably take about 3 small spatulas full: the sodium hydrogen carbonate neutralises the acid)
- ▼ Add 2cm³ Benedict's reagent to the boiling tube and put it back in the waterbath, using the tongs. Boil it again for 3-4 minutes.

If non-reducing sugars (e.g. sucrose) were present in the original sample, the acid will hydrolyse them to monosaccharides, which are all reducing sugars: they will then give a green/yellow/brick-red precipitate with Benedict's reagent.

Method - Samples containing both reducing and non-reducing sugars

If you suspect that a sample contains both reducing and non-reducing sugars:

- ▼ Test with Benedict's reagent and obtain the precipitate as described above (using double quantities of sample and reagent)
- ▼ Filter the tested sample through filter paper, and collect the filtrate.
- ▼ Test the filtrate with Benedict's reagent.
- ▼ Continue this process, using the filtrate each time, until you no longer get a positive result with the Benedict's test.
- ▼ Add 1cm³ dilute hydrochloric acid to the remaining filtrate and boil it to hydrolyse any non-reducing sugars it contains. Neutralise the mixture and re-test with Benedict's reagent.
- ▼ If a non-reducing sugar was present in the original sample there will be a positive result once again.

Method - Iodine in potassium iodide solution test for starch

- ▼ Place a small sample of starch solution on a spotting tile or in a test-tube.
- ▼ Add a few drops of iodine in potassium iodide solution.

The starch will combine with the iodine to give a deep blue-black colour. This test is very sensitive, so only tiny quantities are needed. The colour will fade after a while.

continued

Method - Emulsion test for lipids

This test uses the fact that alcohols will dissolve lipids, but water will not.

- ▼ Place a small sample of lipid in a test-tube. Add $1-2\text{cm}^3$ of alcohol and shake the test-tube thoroughly for a few minutes. Place it in a test-tube rack and allow it to settle.
- ▼ Collect a clean test-tube and half fill it with tap water. Carefully pour the alcohol layer from your sample into the water.

The lipids will dissolve in the alcohol as the sample is shaken. When the solution is poured into the water, the alcohol will dissolve in the water, but the lipids will come out of solution and form fine droplets. Thus if there are lipids in the sample, they will form a cloudy white emulsion in the water.

Method - Biuret test for proteins

- ▼ Place 2cm^3 of protein solution in a test-tube. Add 2 cm^3 of dilute potassium hydroxide solution, then add 0.5cm^3 of dilute copper sulphate solution, a drop at a time. Shake the test-tube gently, then leave it in the test-tube rack for a few minutes.

If protein is present, a purple colour will develop after a few seconds (s). If there is no protein, the solutions will remain very pale blue from the copper sulphate solution.

These methods may be used for testing materials in a laboratory, or for testing foodstuffs to analyse their contents.

Teaching Notes

For the emulsion test, either ethanol or iso-propanol will work, provided it is a strong solution (at least 70%).

The only problem with the standard tests is doing the emulsion test on milk, in which the emulsion effect is masked. The Sudan III test for lipids may be more appropriate for this situation, as it gives a clearer result. If milk is shaken up with Sudan III and methylene blue and allowed to settle, the red Sudan III will separate out in the fatty layer at the top, and the methylene blue in the aqueous layer at the bottom.

The test for reducing sugars can be made quantitative by carrying out the Benedict's test on a series of known concentrations of glucose solution, to produce a colour series, then testing some unknowns to identify where they fit in the series. This could be introduced as a problem for the students, to provide experience in planning experiments.

Clinistix or equivalent can be used to test for different glucose concentrations.

Once the standard tests are understood, you could try varying the procedure, for example, by suggesting that they are testing foodstuffs for a supermarket firm who have been told that some of their foods have been spiked: try adding starch to the milk, or injecting some fats into oranges, for example.

Requirements per student

Boiling tubes
Test tubes
Small measuring cylinders or dropping pipettes
500cm³ beaker
Bunsen burner, tripod and gauze
Test-tube rack
Test-tube tongs
Spotting tile
Small spatula
Filter funnel and filter paper
Safety glasses

Benedict's reagent
Dilute hydrochloric acid
Sodium hydrogen carbonate powder
Iodine in potassium iodide solution
70-80% alcohol solution
Biuret 1% copper sulphate solution
Biuret 15% potassium hydroxide solution

Standard solutions of sucrose (5%), glucose (5%), Starch (1%), albumin (5%) and a small sample of cooking oil.

continued

Chromatography

Introduction

Chromatography is a technique used to separate and analyse which molecules are present in a mixed extract from living material. It depends on the use of organic solvents, which dissolve the molecules concerned. These solutions are allowed to move up a thin layer of inert material, such as paper or a thin layer of silica gel, carrying the molecules with them. The distance the molecules move is related to the size and shape of the molecule, as well as their relative solubility in the solvent and the water associated with the layer of inert material.

For each combination of molecule and solvent, the distance moved relative to the solvent alone is characteristic, and can be used to identify the molecule. The value is known as R_f (Relative front) and is calculated by distance moved by molecule over the distance moved by the solvent.

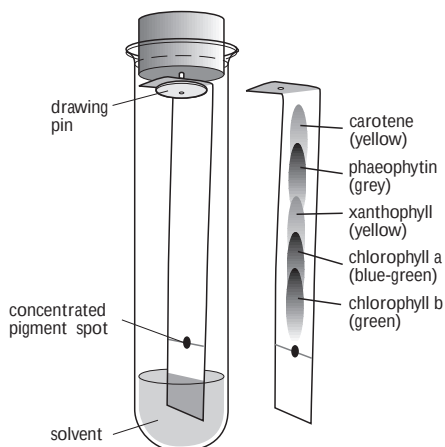
Method

- ▼ Cut a piece of chromatography paper (or filter paper) about 1.5cm wide, and long enough to reach the bottom of the boiling tube. Fold the top 1cm over, and make sure the paper will fit into the boiling tube, with the folded edge at the top, the end nearly reaching the bottom, and the sides NOT touching the sides of the tube. If necessary, trim it to fit.
- ▼ Rule a pencil line (not pen!) across the paper about 2.5 cm from the bottom. Mark the centre of the line with a cross. Write your name at the top of the strip in pencil.
- ▼ Collect a handful of plant material, and cut it up quite finely with a sharp knife. Place 5 cm³ of propanone in the mortar, and grind up the plant material in the mortar, using the pestle and a small amount of sand, (or use a liquidiser). The green pigments should dissolve into the propanone. As you grind up the plant material, remove any tissue that is well ground, and add more, but do not add any more propanone: you need to get as concentrated a solution as possible.
- ▼ Once you have a concentrated solution, filter the contents of the mortar through a piece of gauze in a filter funnel. Discard the solid materials.
- ▼ Put a small amount of solvent (to a depth of not more than 1.5 cm) in the bottom of the boiling tube. Put the bung in the tube and leave it on one side so that the air in the tube can saturate with the solvent vapour.

continued....

- ▼ Using a piece of very fine capillary tubing or the head end of a pin, place a drop of the extract on the cross in the middle of the pencil line of the paper strip. Use a hair dryer to dry the drop, then put another drop in the same place. Repeat this process until you have a small area of concentrated pigment. Be patient with this: you will get a much better result if you let each drop dry properly before you add the next. You should add at least 5-6 drops.
- ▼ Use a drawing pin stuck into the base of the bung, or a split bung, to suspend the paper in the boiling tube, so that the end of the paper reaches into the solvent, but the spot of pigments is not in the solvent. Adjust the amount of solvent if necessary.
- ▼ Leave the boiling tube in a rack to allow the solvent to move up the filter paper. When the solvent has nearly reached the top of the strip of paper, remove the bung and detach the paper. Draw a pencil line on the paper to mark the level reached by the solvent, then leave it on the bench (or in a fume cupboard) to dry.
- ▼ Measure the solvent distance, and the distances moved by the spots of pigment. Identify the pigments by their colour and R_f values, using the information given in the table below. If you have produced a good concentration of pigments in the extract and spot, you should be able to identify all five pigments.

Pigment Name	R_f value	Colour
Carotene	0.95	Yellow
Phaeophytin	0.83	Yellow-Grey
Xanthophyll	0.71	Yellow-Brown
Chlorophyll a	0.65	Blue-Green
Chlorophyll b	0.45	Green

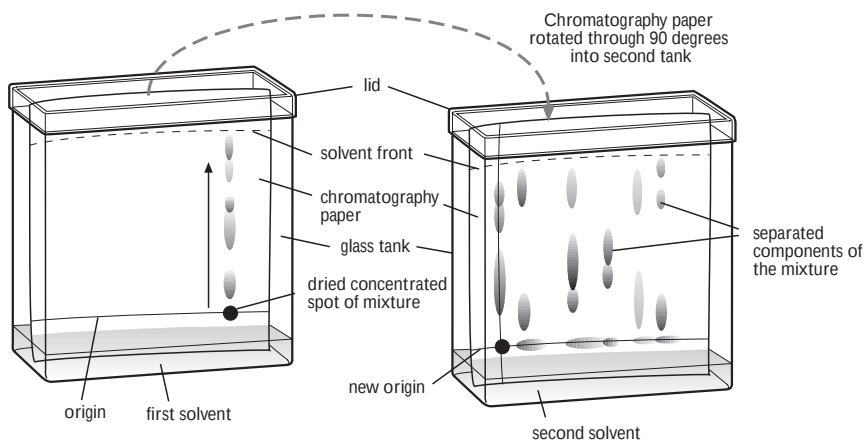


continued

Method - Two way chromatography

Two way chromatography is used to make further separations of substances that may not have been separated sufficiently on the first run of a chromatography investigation.

The chromatography paper from the first run is turned by 90° and re-run as shown in the diagram. Details are provided with Chromatography kits, and are not repeated here as no new principles are involved.



Teaching Notes

The solvent is made from 1 part 90% Propanone (Acetone) to 9 parts Petroleum ether (alternatively 12 parts: 100 parts)

The plant material needs to be as green as possible! Nettle leaves work well, and can be handled easily with gloves. Alternatively, spinach leaves also give a good result. As a possible extension, or for a student project, it might be interesting to try a different coloured leaf, such as red cabbage, although the R_f values for any extra pigments may be difficult to find.

One important factor in getting the practical to work well is to get the chlorophyll extract as concentrated as possible: the students need to spend time over this, and grind up quite a lot of plant material in as little propanone as they can. The other stage at which patience is needed is the preparation of the pigment spot. They must let each drop dry thoroughly, before adding the next, and they need to add at least 5-6 drops. Using a hair dryer speeds up the process. To keep the evidence of the experiment; after allowing the filter paper to dry, it should be sealed under Sellotape so the pigments do not fade.

If you have access to thin layer plates, they tend to work better than paper sheets, but are more complex to set up. If you do use thin layer plates, the R_f values will be different from those given here.

Requirements per student

- Filter or chromatography paper
- Scissors
- Boiling tube with a rubber bung
- Drawing pin
- Ordinary pin or piece of fine capillary tubing (especially fine chromatography droppers are available)
- Pestle and mortar or liquidiser
- Sand for grinding
- Pencil and ruler
- Sharp knife
- Cutting tile
- Filter funnel
- Piece of gauze, about 12 cm square
- 50 cm³ beaker
- Test-tube rack
- 5 cm³ propanone
- 10 cm³ solvent
- Plant material, e.g. nettles, spinach etc.

An investigation into the action of amylase on starch agar plates

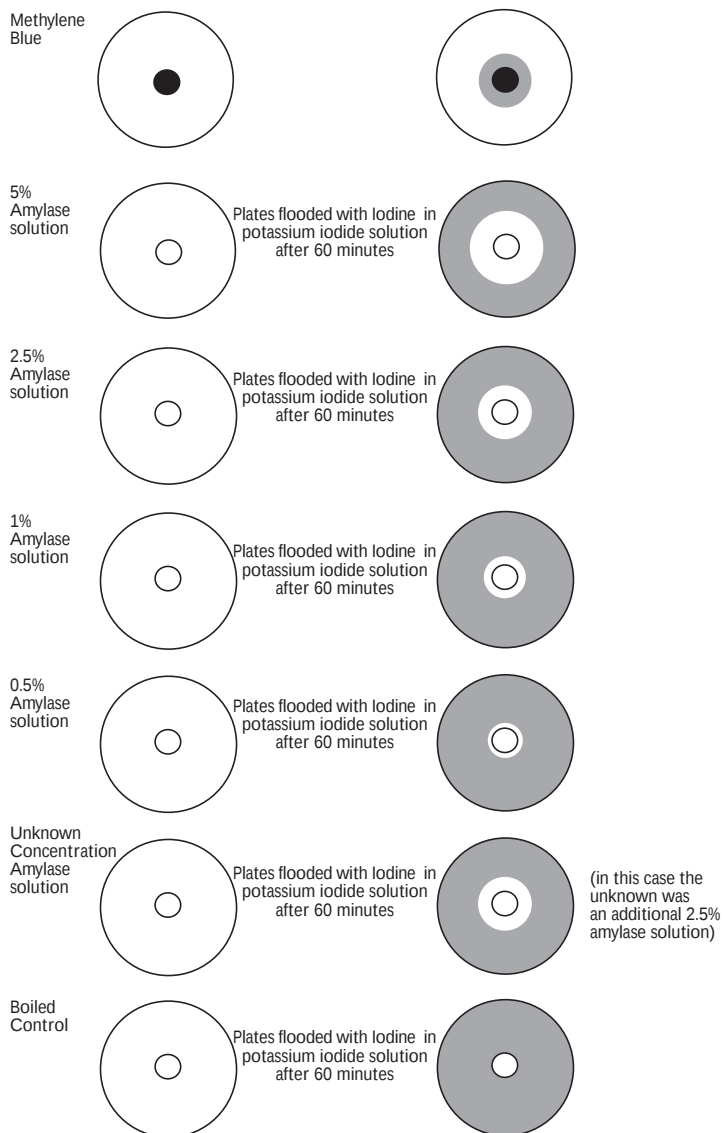
Introduction

Agar plates containing starch are a simple means of estimating the activity of carbohydrase enzymes. The clear zones found after treatment with iodine in KI solution can be used to compare the concentrations (assaying) of amylase in various solutions. Alternatively, it may provide a means of investigating the action of amylases from different sources.

Method

- ▼ Collect 7 petri dishes of starch agar, and label them with your name and the following concentrations of amylase solution: 5%, 2.5%, 1%, 0.1%, Boiled 5% amylase solution control. Also unknown(s), and methylene blue. Do not open the lids until you have to, and then try to work quickly, replacing the lids as soon as possible.
- ▼ Using a sterilised cork borer, make a well in the centre of each plate. Remove the plug of agar with a mounted needle if it does not come out with the cork borer. Put the plates on a side bench where they may be left undisturbed, after the enzyme solutions have been added.
- ▼ Using a dropping pipette or syringe, place a few drops of the 5% enzyme solution in the well in the first plate, be careful not to get any enzyme solution on the surface of the agar, and do not fill the hole too full.
- ▼ Using a clean pipette or syringe each time repeat step 3: with the other known solutions of enzyme. Fill the well of the control plate with boiled 5% amylase solution, and fill the wells of the other plates with the appropriate solutions.
- ▼ Leave the plates undisturbed for at least an hour, do not move them, as you may spill the solutions out of the wells.
- ▼ For all the dishes except the one with the methylene blue, flood the surface of the plates with iodine solution and leave for several seconds until the blue-black colour develops. Carefully pour off any excess iodine solution and gently rinse the agar with water.
- ▼ Examine the plates and measure the diameter of the clear zones around the wells. Recording the diameters of the clear zones produced by the known solutions should allow you to determine the concentration of the unknown solution(s) provided.
- ▼ Observe the methylene blue plate.
- ▼ Describe and explain all the results.
- ▼ Draw a graph of clear zone diameter against concentration of amylase solution, from which unknown concentrations of enzyme could be derived from the diameter of the clear zone they produce.
- ▼ Comment on this method of assaying enzyme solutions of unknown concentration.

continued



Following the time-course of an enzyme-catalysed reaction by the rate of disappearance of substrate using amylase

Introduction

An investigation into the action of amylase on starch in solution.

Amylase catalyses the hydrolysis of starch to maltose (a reducing sugar).

This investigation could be used to follow both the disappearance of substrate and the formation of products.

Method:

- ▼ Using the 0.1% amylase solution, 0.1% boiled amylase solution and the 1% starch solution provided.
- ▼ Measure 5cm³ of the starch solution into each of two boiling tubes labelled A and B. Place a glass rod in each tube. Collect several paper towels or tissues and have them ready.
- ▼ Place one drop of iodine in potassium iodide solution in each well of 3 spotting tiles, until you have all the drops ready. (N.B. Iodine solution fades quite quickly in the light)
- ▼ Add 5cm³ of 0.1% amylase solution to tube A and 5cm³ of 0.1% boiled amylase solution to tube B (at the same time if possible). Immediately start your stopclock and stir the solutions.
- ▼ At 30s intervals, remove a drop of the reaction mixture from each tube using the glass rods, and test them with the drops of iodine solution. Keep one tile just for samples from tube B.
- ▼ Wash and wipe the glass rods thoroughly with a paper towel or tissue, and replace them in the boiling tubes.
- ▼ Continue to test the reaction in this way every 30s, until there is no colour change seen with the iodine solution (achromic point) when mixed with solution A. Record the time taken for the starch to disappear.
- ▼ The remaining solution in tube A can be tested with Benedict's reagent to confirm the presence of reducing sugars.
- ▼ Describe and explain your results, and suggest how you could improve the method used.
- ▼ Plan how you could adapt this experiment to investigate the effect of temperature, pH, substrate concentration, or enzyme concentration on the rate at which amylase breaks down starch in solution.

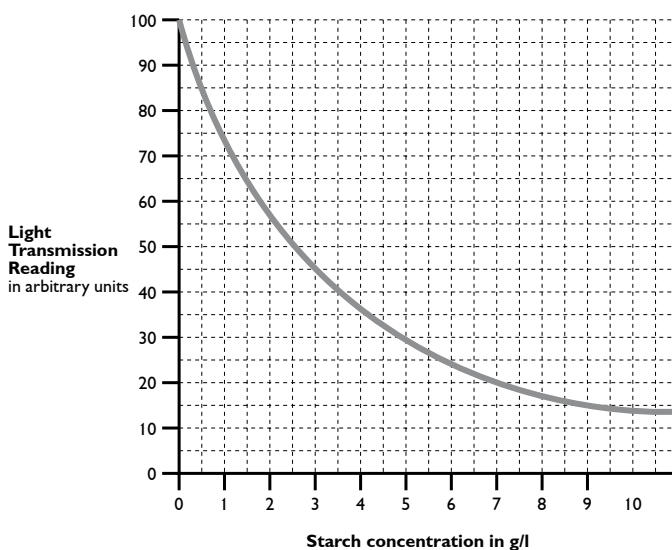
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Teaching Notes

The strength of the starch solution can be adjusted if necessary. A common occurrence is for students to complain that the “experiment is not working”, when what in fact has happened is that the reaction has proceeded so quickly that the first iodine test is negative, and of course remains so.

The practical is carried out at room temperature, but each group of students could work at a different temperature.

The concentration of starch in a solution can be determined by reacting starch with iodine solution and measuring the depth of the blue / black colour produced, with a colorimeter. This can be quantified by reference to a calibration curve plotted from colorimeter readings of a series of known concentrations of starch solution.



Calibration curve of colorimeter reading against starch concentration in g.dm^3

The presence of maltose can be confirmed by carrying out a Benedict's test after the reaction is complete to check for reducing sugars. (Ideally all solutions should be tested for reducing sugars before starting the experiment, although it can be assumed that they are uncontaminated. However, enzyme powder has been known to be contaminated with reducing sugars in the 'packing' powder which is added to increase the ease of 'flow' in dispensation.)

continued

Requirements per student

2 boiling tubes
Labels or marking pen
Stopclock
Test-tube rack
2 glass rods
Paper towels or tissues
3 spotting tiles
Dropping pipettes
2 x 10cm³ measuring cylinders or syringes
Water bath

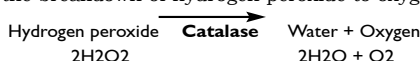
Iodine in potassium iodide solution (this can deteriorate with storage)
10cm³ 0.1% amylase solution and 10cm³ 0.1% boiled, cooled amylase solution
20cm³ 1% starch solution
Benedict's reagent
Distilled water

ADDITIONAL ENZYME EXPERIMENTS

Experiment to investigate the effect of temperature on the activity of the enzyme catalase

Introduction

Catalase catalyses the breakdown of hydrogen peroxide to oxygen and water.



The rate of production of oxygen can be measured, and gives a measure of the rate of the reaction.

Catalase is produced in cells to prevent the accumulation of hydrogen peroxide, which is toxic.

In this experiment potato tissue is used as a source of catalase.

The effect of temperature on the rate of this reaction will also be investigated.

TAKE CARE with the HYDROGEN PEROXIDE - wear safety glasses and try not to get any on your skin, wash off immediately if you do.

Method

▼ There are various pieces of apparatus that you can use to collect and then measure the volume of oxygen gas that will be evolved when the catalase in the potato tissue reacts with the hydrogen peroxide.

a The O_2 gas can be collected over water in an inverted measuring cylinder.

b The O_2 can be collected over water in the inverted barrel of a 20 cm^3 syringe.

c The O_2 can be collected and measured in a manometer tube filled with coloured fluid, linked to the reaction vessel. You will be advised which method(s) to use.

The details below relate mainly to the 'inverted measuring cylinder' method.

▼ Using a cork borer a little smaller in diameter than your boiling tubes, cut some long potato cylinders. Using a knife (scalpel) carefully cut the potato cylinder into discs about 1mm thick.

▼ Place the discs into a few cm^3 of pH7 buffer in a watch glass to keep them moist. You will need 50-60 discs.

▼ Using a boiling tube as the reaction vessel, place about 10 potato discs into the tube and just cover them with a measured volume of pH7 buffer.

continued

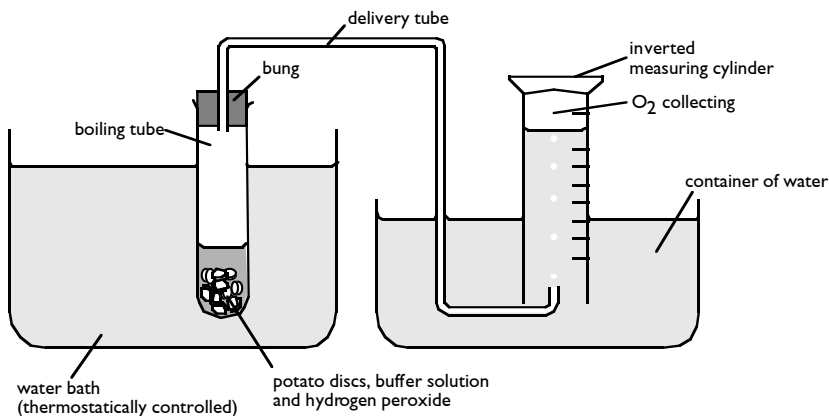
- ▼ Place the tube in a water bath, at the first temperature you intend to use, e.g. 20°C and allow the discs time to reach the correct temperature. Place a boiling tube of hydrogen peroxide into the water bath at the same time, so it also reaches the same temperature.
- ▼ Assemble your apparatus; using a rubber bung with a delivery tube inserted, and check that this will fit under the inverted 25 cm³ measuring cylinder full of water in its container of water. You may have to use a clamp stand to support the cylinder.
- ▼ When you are ready, measure 5 cm³ of hydrogen peroxide (H₂O₂) in a syringe. Carefully loosen the bung of the boiling tube, and very quickly and safely add the peroxide. Replace the bung and start your stopclock, tap or shake the tube if necessary to mix the contents.
- ▼ Record the volume of oxygen produced every 30 s for 3 minutes.
- ▼ Remove the boiling tube, wash it out and add 10 new discs and the same volume of pH7 buffer as used before. Put the tube back in the water bath and carefully raise the temperature by 10°C.
- ▼ Refill the measuring cylinder with water (if necessary).
- ▼ Add 5 cm³ hydrogen peroxide to the boiling tube, quickly replace the bung, start the stopclock and take your readings as before.
- ▼ Repeat your experiment at 30, 40, 50 and 60°C using a new set of potato discs each time.

Look at the rate at which the oxygen has been produced during each 30 second period. Can you explain your results in terms of an enzyme reacting with its substrate?

Calculate the mean amount of O₂ produced during the 3 minute period, at each temperature. Plot a graph of these figures against temperature.

Explain how you could improve your method to obtain more reliable results.

Plan how you could adapt this experiment to investigate the effect of pH, substrate concentration, or enzyme concentration, on the action of Catalase. Check your new plan with your supervisor, then carry out your investigation and write a full report including analysis of results, conclusion and evaluation etc.



Teaching Notes

This practical usually works quite well and it is a good exercise for students to demonstrate their manipulative skills. A number of methods can be used in this experiment to measure the volume of oxygen produced, as mentioned on the student sheet: e.g. over water in an inverted measuring cylinder, over water in the barrel of a syringe (a small piece of rubber tubing is attached to the narrow end of the syringe which has a screw clip so the barrel can be filled with water) or in any simple manometer.

A bit of juggling with the number of potato discs and the volume of peroxide (or its concentration) may be necessary to achieve a good result. But students using the inverted measuring cylinder method at 40°C recently obtained results of:

O₂ produced: 30s/5 cm³, 60s/2 cm³, 90s/1.5 cm³, 120s/1.0 cm³, 150s/0.5 cm³, 180s/0.5 cm³.

Hydrogen peroxide can be added from a syringe, the needle of which is inserted through the cork, to improve accuracy.

If the students are going on to measure the effects of pH, they will need to be supplied with quantities of buffers: pHs suggested are 4, 5, 6, 7, 8, 9. They will need only a few cm³ of each buffer per student.

Substrate concentration can be varied using different concentrations of hydrogen peroxide, such as 5 vol., 10 vol., 15 vol., and 20 vol.

Effect of enzyme concentration can be measured by varying the number of potato discs used. Alternatively you could vary the size of the discs (either diameter or thickness). It is quite important that the discs are cut to the same sizes for this version of the experiment. This would also allow them to consider the effect of surface area to volume ratio.

continued

Requirements per student (measuring cylinder method)

Boiling tube
Large beaker (500 cm³)
Bunsen burner, 2 tripods and gauzes or
thermostatically controlled water bath 1 tripod & gauze
Bung with delivery tube
Measuring cylinders 25 cm³ and 10 cm³
Trough or equivalent
Cork borer 10 mm diameter (No6)
Watch glass
2 100 cm³ beakers
5 cm³ syringe or dropping pipette
1 clamp stand
Stopclock
Thermometer
Knife/scalpel
50 cm³ pH 7 buffer
50 cm³ 20 volume Hydrogen peroxide
Potato

Experiment to investigate the effect of pectinase enzyme on the extract from apples

Introduction

Pectinase is used extensively during the production of commercial fruit juices, especially apple juice, which can be quite difficult to extract from some varieties of apple. The apples are pulped and heated to 30°C before the enzyme is added. After up to 2 hours of pectinase treatment (the time depends on the type of apple and the dosage of enzyme), the pulp is squeezed. The yield of juice can be increased by as much as 20%.

Commercial pectinases are obtained from fungi.

Pectinase breaks down the pectin of the middle lamella which occurs between the walls of the fruit cells. Otherwise the pectin forms a gel which slows the release of the cell contents.

Method

- ▼ Collect two medium-sized apples of different varieties and chop them (peel, core and all) into fine pieces.
- ▼ Weigh the material produced by each apple and then divide each sample into two equal portions.
- ▼ Place the four samples into beakers and label them carefully; e.g. Variety 1 with A and B; and variety 2 with C and D.
- ▼ Add 2 cm³ of pectinase solution to samples A and C, and 2 cm³ distilled water to B and D. Mix the contents of each beaker with a clean glass rod.
- ▼ Place the beakers into a water bath set at 35-40°C and incubate for about 20 minutes.
- ▼ Prepare four filter funnels lined with coffee filter paper and filter the contents of each beaker into measuring cylinders. Leave until all the juice has dripped through.
- ▼ Note the volume of apple juice which has collected in each of the four cylinders after 5, 10, 15 minute intervals.
- ▼ In your conclusion compare and explain the results from beakers A/B, and C/D; and then A/C & B/D.
- ▼ Suggest improvements to the method that you have used.

Teaching Notes

The pectinase enzyme can be obtained from NCBE, Dept. of Microbiology, University of Reading, Whiteknights, Reading, RG6 2AJ. It should be diluted 50:50 with water before immediately before use.

The practical can also be used as the basis for project work, by investigating the conditions used for extraction, e.g. volume of enzyme or incubation temperature. An additional twist is to incubate the juice for 15-20 minutes before adding the enzyme: this is done in industry to allow enzyme inhibitors in the pulp to oxidise and should enhance the extraction rate. The extraction can be compared with and without the waiting time.

Requirements per student

- A chopping board
- A sharp knife
- 4 × 100 cm³ beakers
- 2 × 10 cm³ measuring cylinders
- 4 × 50 cm³ measuring cylinders
- 4 filter funnels, with filter papers
- 4 glass stirring rods
- Water bath set at 35-40°C
- Balance
- Stopclock
- 4 cm³ pectinase solution (diluted)
- 4 cm³ distilled water
- 2 medium-sized apples (different varieties)

Experiment to investigate the action of Immobilised Lactase enzyme on Lactose in milk

Introduction

Immobilised enzymes and cells are used increasingly in biotechnology, as they allow easier processing and harvesting of products. The enzyme or whole cell preparation is mixed with a solution of a gel based on alginate or a similar polymer, usually extracted from seaweed. Once they are mixed, the gel is treated to make it set. The beads or mesh can then be used in a column or similar flow system.

Using enzymes in this way has a number of advantages:

The beads are simple and rapid to produce; the process can be carried out under sterile conditions if necessary, so production can be kept clean

The process is gentle on the enzymes, causing little damage

It stabilises the enzymes, making them more resistant to heat, pH changes etc.

The enzymes can be used and re-used for long periods

Harvesting is simple, as the beads can be removed from the substrate and products easily, for example by sieving

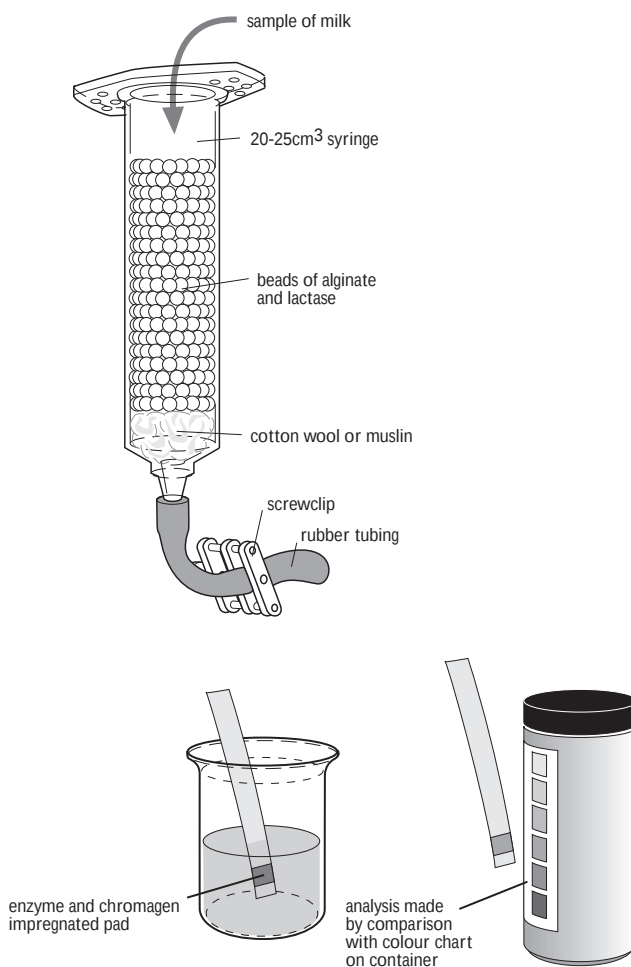
If the beads or mesh are placed in a column system, a continuous flow process can be set up, which makes production more economic.

Method

- ▼ Collect 20 cm³ of 2% sodium alginate solution.
- ▼ Stir it with a glass rod to ensure an even consistency.
- ▼ Dissolve 1.4g calcium chloride in 100 cm³ distilled water.
- ▼ Add 2 cm³ of lactase solution to the alginate gel, and stir to mix.
- ▼ Draw some of the alginate mixture into a syringe. Expel it drop by drop into the calcium chloride solution, from a height of about 10 cm: this will allow it to form beads.
- ▼ Leave the beads to stand in the calcium chloride solution for 10 minutes, to allow the beads to set completely.
- ▼ Drain the beads through a sieve or tea strainer and rinse with tap water.
- ▼ Place a small piece of cotton wool or muslin cloth in the bottom of a 20 cm³ syringe (or larger) to prevent the beads blocking the nozzle. Place a short piece of rubber tubing over the nozzle of the syringe and put an efficient screwclip over the tubing. This will allow you to control the flow of milk through the column.

continued

- ▼ Support the syringe in a clamp stand, nozzle downwards, and fill it with the alginate beads containing lactase, to within 1cm of the top. (See diagram)
- ▼ Collect a 50cm³ sample of fresh milk, and transfer 5 cm³ into a test tube. Label it 'No run' and put the test tube in a rack. Pour the rest of the sample slowly through the column, collecting the treated milk in a clean 50cm³ beaker at the bottom.
- ▼ Take a 5cm³ sample of the treated milk, put it in a test tube and label it 'Run 1'. Slowly pour the rest of the milk through the column again, and collect in a beaker.
- ▼ Take a new 5cm³ sample, label it 'Run 2', and repeat the process once more. Collect a final sample of treated milk and label it 'Run 3'.
- ▼ Test each of the four milk samples you have taken with a Clinistix strip, to measure the quantity of glucose in each of the samples.



Teaching Notes

The alginate solution and the calcium chloride solution can be prepared in advance, so the students can start making the beads immediately. Also, 3-4 sieves will usually be enough for a whole class.

The lactose solution is quite expensive, but it needs to be used neat, as supplied,

This practical works well, and the students usually enjoy making up the beads. A similar process can be used to prepare other immobilised enzymes e.g. sucrase (invertase), or whole cell samples e.g. yeast.

This experiment would make a good basis for a student project, for example trying different quantities of enzyme, or different sizes of beads to get the optimum efficiency in a simple column. They could also try different temperatures or using different pH-buffered solutions, to explore the improved stability of immobilised enzymes over non-immobilised ones.

Requirements per student:

- Glass stirring rod
- Sieve or tea strainer
- 5 or 10 cm³ syringe
- 20 or 25 cm³ syringe with rubber tubing and screw clip
- Muslin
- Clamp stand
- 4 test tubes
- Test tube rack
- 1 x 250 cm³ beaker
- 2 x 100 cm³ beakers
- 3 x 50cm³ beakers
- Labels or marking pen
- 20 cm³ of sodium alginate solution
- 1.4g calcium chloride
- 2cm³ Lactase solution (as supplied).
- Distilled water
- 4 Clinistix (or similar) test sticks for glucose
- 50 cm³ sample of fresh milk

GAS EXCHANGE WITH THE ENVIRONMENT

An experiment to investigate gas exchange of seeds using a simple respirometer

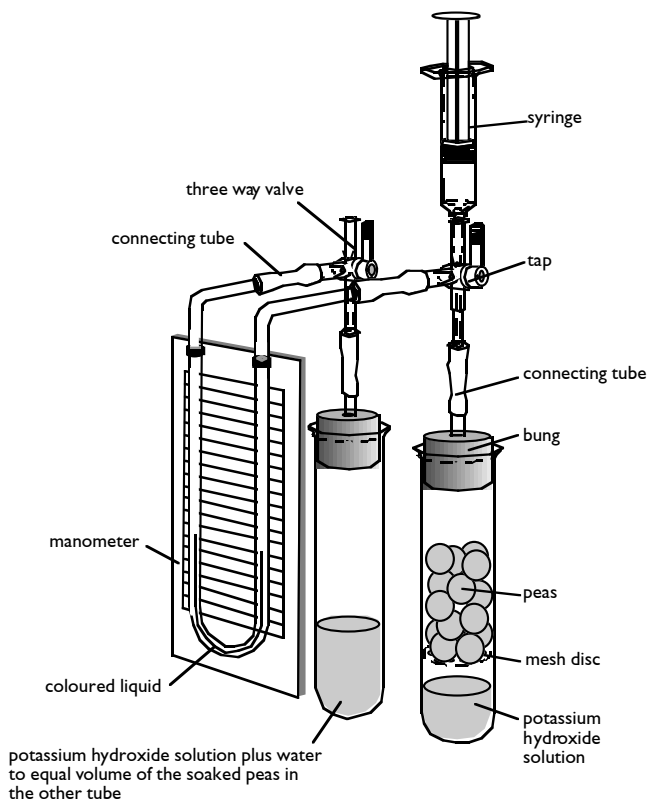
Introduction

A respirometer is an apparatus used to study respiration, rates of respiration and respiratory quotients in whole organisms, e.g. soaked, germinating seeds such as peas; small animals, e.g. woodlice, mealworms, blowfly larvae, earthworms and locusts; and microorganisms, e.g. yeast.

The principle of the apparatus is that the organisms respire in a closed chamber, oxygen is taken up and an equal volume of carbon dioxide is given off. The carbon dioxide is absorbed by a carbon dioxide absorber (e.g. potassium hydroxide), the volume of gases in the chamber decreases, and the proportional decrease in pressure can be measured by a manometer.

- ▼ To measure the rate of respiration (to obtain a quantitative result), the oxygen uptake over a certain period of time is measured.
- ▼ The potassium hydroxide solution (or soda lime pellets) absorbs the carbon dioxide produced by the organism, as the pressure within the apparatus falls the liquid in the manometer moves towards the chamber with the living organisms.
- ▼ The rate at which the liquid moves towards the chamber is equivalent to the rate at which the organisms are taking up oxygen (rate of aerobic respiration).
- ▼ The air in the experimental tube is sensitive to changes in temperature, and these changes could interfere with volume and pressure changes due to the respiration of the organisms. Therefore a second tube is attached to the other side of the manometer which acts as an 'equalising chamber' and compensates for any changes in volume and pressure within the experimental tube as a result of changes in external temperature. Any change of external temperature will affect both tubes equally so any effect on the chamber containing the organisms is cancelled out.
- ▼ A control apparatus should also be set up which contains inert (non-reactive) material or dead organisms equal in mass to the living organisms in the experimental apparatus. This is necessary to demonstrate that any changes in the fluid level in the manometer of the experimental apparatus is due solely to the respiration of the living organisms.
- ▼ (One control apparatus may be sufficient for the whole group. If you are setting up a control, you will need the same apparatus as below, but with an equivalent mass of dead organisms, e.g. boiled, cooled peas, or inert material, in the experimental tube).

continued



- ▼ Both sets of apparatus should be set up in a water bath, with the manometers positioned over the edge so that they are not in the water. Water is less prone to changes in temperature than air, and the water bath allows a constant temperature to be maintained throughout the experiment. It also enables the temperature to be changed if the effect of temperature on the rate of respiration is to be investigated.

In this experiment the rate of respiration of a living organism will be investigated.

The affect of temperature on the rate of respiration can also be investigated.

Method

HANDLE THE MANOMETER VERY CAREFULLY as the column of coloured fluid easily breaks up (in fact you will be doing very well if it doesn't).

- ▼ To the experimental boiling tube add about 5 cm³ of potassium hydroxide solution (or a known mass of soda lime pellets). Insert the mesh disc and add a known mass of soaked peas (the peas must not come into contact with the chemical)

continued

- ▼ To the second boiling tube (the thermobarometer or compensating tube) add the same volume of KOH solution or mass of soda lime pellets used before PLUS some distilled water equivalent to the volume of the soaked peas and the mesh disc in the experimental tube.
- ▼ Firmly push the two rubber bungs into the boiling tubes, and very carefully insert the manometer into the appropriate connectors on the plastic taps inserted in the rubber bungs (it may be necessary to grease these connections).
- ▼ Make sure the taps are both in the 'open to the air' position.
- ▼ Very carefully transfer the apparatus to a water bath, tubes inside and manometer outside.
- ▼ Allow the apparatus to come into equilibrium at 20°C for about 10 minutes.
- ▼ Turn the plastic taps to the 'air to manometer' position.
- ▼ Set the syringe at 0.5 cm³ and insert it into upper part of the plastic tap above the experimental tube.
- ▼ Use the syringe, very carefully to adjust the levels of the manometer fluid, so it is equal in the 2 arms. Note the position of both fluid levels and the new reading on the syringe.
- ▼ Turn both plastic taps to the 'manometer to boiling tube' position.
- ▼ The apparatus should now be ready to use.
- ▼ Record the position of the manometer fluid at about 2 minute intervals for 16 minutes (But watch carefully and be prepared to change your timings according to what is happening in your experiment). Record your results.
- ▼ At the end of your first set of readings - adjust the tap above the experimental tube to the 'air to manometer' position and very carefully use the syringe to reset the position of the manometer fluid and note the new syringe volume. If possible repeat your readings for a second and third time and record your results.
- ▼ Look at the control experiment and record the results.
- ▼ If you are able to continue your procedure to investigate the effect of temperature on the respiration rate, increase the temperature of the water bath to 30°C. Allow the apparatus to equilibrate at the new temperature, reset the manometer fluid with the syringe, and repeat your readings, and record your results
- ▼ Increase the temperature to 40°C or drop it to 10°C, and repeat your readings and record your results. (With invertebrate specimens you would probably restrict the temperatures to between 10 - 20°C.
- ▼ Work out the volume of O₂ consumed/hour:
$$\text{O}_2 \text{ consumed} = \text{Volume of O}_2 \times 60 / \text{Time in mins} \times \text{mass of living organisms (g)} = \text{cm}^3 / \text{g} / \text{hr}$$

Plot a graph of volume of oxygen consumed/time

Analyse all your results, including those from the control experiment, write your conclusions, and note any improvements you would make in the method used.

Teaching Notes

This experiment can be disappointing if the fluid does not move, or even worse, moves in the 'wrong' direction. Students must be ready for this and be prepared to check the apparatus and keep resetting the manometer fluid etc.

It is suggested students work in pairs because some of the manoeuvres are quite difficult.

You may not have the apparatus described.

However the method used does cover all the main principles involved in the use of any Respirometer. Detailed instructions will be included by any supplier for use of their particular design of apparatus.

It is suggested that the manometers are half filled with fluid before the lesson starts, as to get the fluid in place without air bubbles, can waste a lot of valuable time. There are complicated ways of introducing the fluid, but usually with care it can be done with a syringe.

One control experiment will probably be enough for the whole group.

Timing of readings should be varied according to the conditions on the day, e.g. type of organism used.

It may not be possible for everyone to investigate the effect of temperature on respiration rate, during the same lesson, but if a few pairs of students have the experiment working well, they could investigate different temperatures and then results can be distributed to the group as a whole.

The volume of O_2 consumed per hour could be calculated:

$$O_2 \text{ consumed per hour} = \text{Volume of } O_2 \times 60 / \text{Time in mins} \times \text{mass of living organisms (g)} \quad (\text{cm}^3/\text{g/hr})$$

A graph can be plotted Volume of O_2 consumed/time

A graph can be plotted Volume of O_2 consumed/time, for different temperatures

Calculate Q_{10} - how does the rate increase for a 10°C rise in temperature

$$Q_{10} = \text{Rate of Respiration at } T + 10^\circ\text{C} / \text{Rate of Respiration at } T$$

The Respiratory Quotient (RQ)

$$RQ = \text{CO}_2 \text{ evolved} / O_2 \text{ consumed}$$

The RQ is obtained using the apparatus described above with water instead of KOH.

If the manometer meniscus does not move, the volume of CO_2 being evolved is the same as the volume of oxygen being absorbed, and the $RQ = 1$

If the manometer meniscus moves away from the seeds more CO_2 is being evolved than oxygen consumed and the $RQ = >1$

If the manometer meniscus moves towards the seeds more O_2 is being absorbed than CO_2 is being evolved, and the $RQ = <1$

It is possible to compare the RQ of seeds soaked for different lengths of time or containing different food reserves.

continued

Requirements for each student

Manometer and manometer fluid (**Sudan III** in paraffin oil/Brodie's fluid)
Two bungs fitted with connecting tubes and adjustable plastic taps (or connecting tubes with rubber tubing and 2 screw clips)
Thermostatically controlled water bath or large beaker, tripod, gauze and Bunsen burner.
Two boiling tubes and 1 mesh basket/disc
Thermometer
Spatula
Stopclock
Balance
25 cm³ measuring cylinder
1 cm³ syringe (a larger one can be used but this will probably have to be supported)
Ice cubes if necessary
Vaseline
15% KOH solution / Soda lime pellets
Distilled water
Specimen material, e.g. peas soaked for 24 hours.

For I control experiment

Similar apparatus
Boiled cooled peas

Gas Exchange and Exercise in Humans

Sample Prior Informed Consent Form

Title of Investigation/Experiment:.....

Objectives:.....

Description of Procedure:.....

Questions: All questions relating to the investigation or experiment will be answered in full.

Safety: All the procedures to be used in this investigation/experiment are widely and commonly used in Schools and Colleges, and although all activity involves some risk of an accident, the risk of harm in this investigation/experiment is no greater than in normal everyday life, or than in a supervised practical lesson.

Right of Withdrawal: You are absolutely free to withdraw from the investigation/experiment at any time. If during the investigation/experiment you feel in any way uncomfortable and wish to stop, you should do so.

Reassurance of Confidentiality: All information obtained as a result of the investigation/experiment will be treated confidentially.

Availability of Results: The data may be recorded manually, and/or stored on a computer, but not in association with your name. You are entitled to a copy of your own results on request.

Use of Results: The data may be used in discussions and/or as part of assignments or projects, but not in association with your name. They will only be used to illustrate general physiological principles, and will not be used for diagnostic or any other purposes

Statement of Consent: I fully understand the nature of the tests, and agree to participate in this investigation/experiment. To the best of my knowledge I suffer from no illness or condition that will:

- i prevent me from successfully completing the tests,
- ii be made worse in anyway by my taking part in the tests.

Name of Subject:.....

Signed:.....

Date:.....

Name of Parent or Legally Authorised Representative:

.....

Signed:..... **Date:**.....

Measuring the Resting Pulse Rate

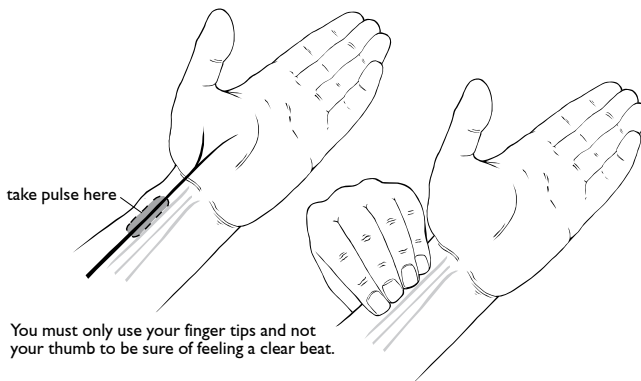
Introduction

The pulse rate gives a direct measure of the heart rate.

It can be felt at the wrist and the neck. Some claim that feeling the pulse at the neck can influence the rate, although the practice is still widespread amongst athletes and coaches.

Method

The pulse can be felt in the radial artery at the wrist as shown.



- ▼ Allow the subject to settle for two minutes (whilst standing).
- ▼ During this time locate the pulse in the left wrist as illustrated (do not use your thumb).
- ▼ After 2 minutes count the pulse beats for 30s, and record the result in a suitable table.
- ▼ It is possible to count for a shorter period e.g. 6s, but when measuring the resting pulse rate the longer the counting time, the more accurate is the conversion to beats per minute (*bpm*)
- ▼ At the end of counting, leave the subject at rest for a further 2 minutes (whilst standing).
- ▼ Repeat the 30s pulse count procedure, and record the result.
- ▼ Allow to rest for 2 minutes (whilst standing).
- ▼ Repeat the 30s pulse count procedure, and record the result.
- ▼ Convert each result to beats per minute and work out the average resting pulse rate per minute.

Teaching Notes

Generally the resting pulse rate is taken when the subject is flat on their back. However, where the intention is to investigate the effect of exercise on the pulse rate, it may be better to measure the 'resting' pulse rate standing, otherwise there is an increase in the pulse rate associated with getting up to participate in the exercise.

Strictly speaking it is not possible to obtain the true resting pulse rate in a classroom or a laboratory, as the pulse will tend to rise as soon as any attention is focused on an individual, so students could be given instruction in taking their pulse rates as they wake in the morning. Taking this truly resting pulse on, say, four consecutive mornings and then taking a mean is a satisfactory method. Resting heart rate can be taken as an indicator of the subject's status, for example some advise that it is best not to train if the resting heart rate is 10% above the normal rate for that person.

In recent years, electronic heart rate monitors (HRM's) have become more readily available. Clear advantages offered by HRM's over manual pulse-taking are that heart rate can be continuously measured over extended periods of time, and also during a wide range of physical activities which do not lend themselves to periodic bouts of wrist holding. The range of the transmitter is about 1metre; this is not a great distance but it does not usually create any practical problems. The receiver, depending on the model and the cost, may give a simple digital display of current heart rate, or it may be capable of storing heart rate data in a number of files which can be retrieved either manually or down-loaded to a PC.

Requirements per student

Stop clock / watch

Sample plan to investigate the effects of exercise on the body.

Method

- ▼ Before you start exercising, make sure that all members of the group have submitted a prior consent form.
- ▼ Subject A should rest for 2-3 minutes, standing, then other members of the group check the subject's resting pulse in beats per minute, and breathing rate in breaths per minute. It is best to check resting pulse and breathing rate three times for each individual, and calculate the average. Breathing rate can be obtained by resting one hand lightly on the subject's back just below the shoulder. (One breath = one inspiration and one expiration.)
- ▼ Subject A should then exercise at a steady rate for three minutes. This exercise can be using an exercise bike, or jogging on the spot, or stepping up and down using a box or the stairs. It is important that the exercise should be at a steady rate, which is one that the entire group can keep up with: it is probably a good idea if the least fit member of the group goes first.
- ▼ Immediately after the exercise, B should count subject A's radial pulse rate. At the same time student C should check the breathing rate, while D keeps track of the time and records the results. Count both pulse rate and breathing rate for 30 s, then multiply the results by two. Subject A should not try to count either pulse or breathing themselves, as this can influence their results.
- ▼ Continue to count pulse rate and breathing rate for 30 s of each minute for three minutes, while subject A rests (standing). Do not stop the clock between measurements; by counting for only 30 s you should have time to record the results for each minute before you start the next.
- ▼ Change roles within your group, and repeat the process (steps 2-5) for each student in your group, so you have all exercised, and recorded results etc.
- ▼ If you have time, repeat the whole process again for all members of your group, to check the reliability of your results.
- ▼ It is also relatively easy to sample exhaled air at various intervals throughout the procedure and to measure the increase in carbon dioxide production with exercise. A method is set out below.

In your report, present the data in suitable ways, and consider the different results from members of your group, see if you can relate them to different levels of fitness.

Evaluate your evidence and your method.

Requirements per group

Step boxes, bench, safe stairs, or exercise bike
Stopclocks

Safety Considerations

General safety considerations when dealing with exercising subjects apply.

Teaching Notes

Although the specifications are worded so that Candidates should be able to **design** and carry out such experiments, they will obviously need some guidelines and be aware of some basic techniques, procedures and general principles; armed with which they will be able to design their particular experimental approach.

To investigate the effects of exercise on the body it is necessary to establish a standard rate of exercise, during and immediately after which it is possible to record various measurements of the effect of exercise.

The validity and reliability, and relative safety of sub-maximal and maximal tests should be understood.

The ability to extrapolate from sub-maximal results to maximal results, and the correlation between various measures of body response to exercise, will all ensure a scientific approach to the investigation.

A cycle ergometer, heart rate monitor, and stethograph allow accurate and reliable recording, but are beyond the reach of many. For those without access to these, the **Step Test**, along with simple measuring of e.g. heart rates, pulse rates breathing rates, temperature, and even composition of expired air (procedure attached) can still produce reasonably valid and reliable results.

Sub-maximal tests like the Step Test pose less risks for the subjects and the responsible supervisor than do maximal tests like the shuttle run, or those that can be performed on the cycle ergometer.

The step test involves stepping up and down a measured height at a controlled rhythm, requires the minimum of apparatus and space, and can be administered to large numbers at the same time. The stepping height can be adjusted for individuals, for example from between 10 cm and 50 cm, depending upon the height and weight of the subject. The stepping rhythm is established with the aid of a pre-recorded signal, of so many steps per minute (*22 for females and 24 for males*). There is always the risk of stumbling, so care must be taken.

The pulse rate and breathing rate can be taken before, during and after a standard stepping period (*usually 3 or 5 minutes*). Once the heart rate has returned to its pre-exercise resting rate, the work period can be repeated if necessary with increased effort, for example by increasing the mass with added weights, and/or the rate of stepping.

Step tests are theoretically as valid (*they should measure what they claim to measure*) and reliable (*they should do so consistently*) as cycle ergometer tests. However, they rely on doing work by raising the body weight against gravity at a set rate, and their validity and reliability depends on correct stepping technique which is difficult to maintain. Any change in the height due to poor leg extension, or in the rate of stepping during a test, will change the work rate, usually reducing it, and this will invalidate the results. Stepping efficiency is also affected by the length and proportions of the legs.

An investigation into the effect of exercise on the heart rate (pulse rate) and breathing rate

Introduction

The heart rate and breathing rate both increase during exercise to supply the muscles with extra oxygen and glucose, and to remove carbon dioxide.

The pulse rate is the product of the heart rate, and can be taken to be the same thing. As the ventricles contract blood is forced out into the arteries, the walls of which expand to accommodate the surge of blood from the heart. When the ventricles relax the elastic walls of the arteries recoil until the next ventricles contract again in the next cardiac cycle.

How the heart rate and breathing rate respond to exercise can be investigated in different individuals. Groups of 4 are best for this activity.

Method

Design an experiment to investigate how exercise affects the heart rate and breathing rate. You will need to consider safety, controls, type of exercise, how to measure the effect on your subject(s), and how you intend to record and analyse your results.

When you have planned your experiment, write out the details, and ask your supervisor to check them before you start the practical.

Carry out the practical investigation when your method has been checked.

Record and analyse your results.

State your conclusions, and evaluate your evidence and your method.

The Estimation of Vital Capacity of Lungs using simple apparatus

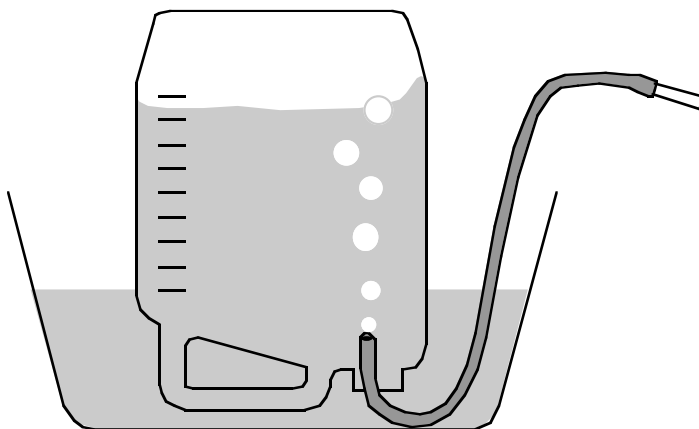
Introduction

SPECIAL SAFETY NOTE

Experiments involving unusual ventilation of the lungs can be dangerous to those suffering from asthma, bronchial disorders of any kind, or epilepsy. Supervisors should be in possession of a Prior Consent Form, and be aware of the status of their subject's health, possible consequences arising from any condition, and the necessary First Aid procedures. The procedures described below are guidelines, the safe implementation of which remains the responsibility of the Supervisor.

Method A - Simple container

- ▼ Collect a transparent container with a single opening, which should have a capacity of at least 6 litres.
- ▼ Using a 500 cm³ measuring cylinder or beaker, measure out 500 cm³ of tap water. Pour this water into the vessel, and, holding the vessel as level as possible, mark the level of the water meniscus using a marker pen.
- ▼ Add another 500 cm³ of water, and mark the new level. Continue adding water in 500 cm³ volumes, until the vessel's capacity is marked off in half-litres to a volume of 5-6 litres.

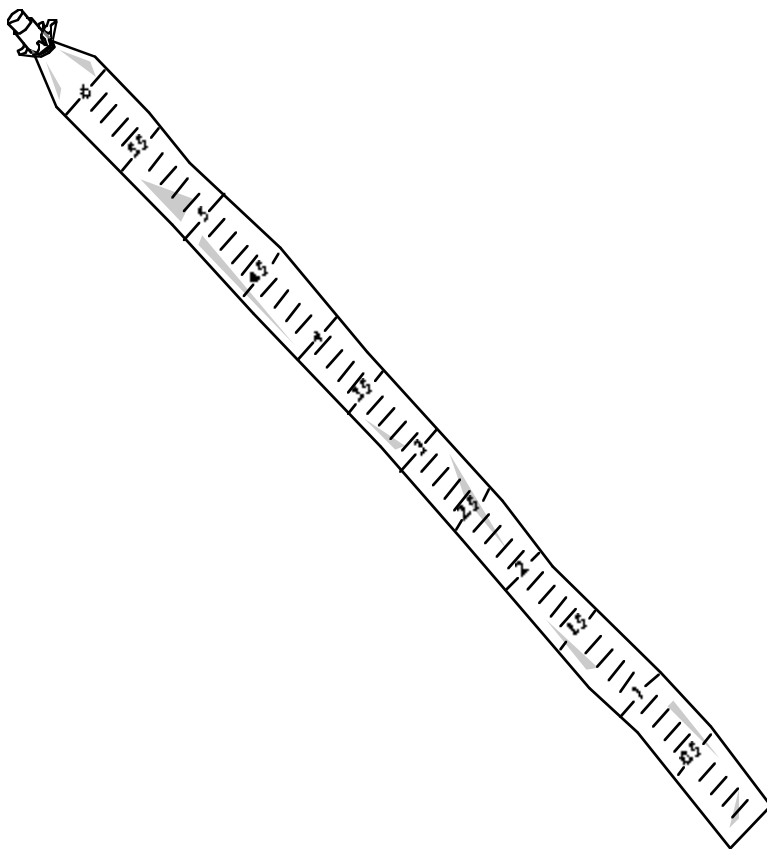


continued

- ▼ Empty the vessel out into a large sink. Fill the sink with water, then fill the vessel. Place a piece plastic sheet or the screw cap over the open end, and carefully invert the vessel in the sink of water so that it itself remains full of water. Remove the piece of plastic sheet or screw cap.
- ▼ Insert a piece of sterile tubing into the vessel, inhale deeply, and blow your exhaled air into the vessel until you have exhaled as far as you can (making sure that you are not losing any air down your nose), displacing the water from the container. Use the scale you have marked on the vessel to estimate your vital capacity.

Method B - Plastic Bag

- ▼ Collect a short length of sterile plastic tubing, an elastic band, and a long plastic bag with a sealed end and a scale printed on it.
- ▼ Place the short tube in the open end of the bag, gather the plastic round the tube and hold it in position with the elastic band, making a tight seal.
- ▼ Now inhale deeply, and exhale through the sterile plastic tubing into the bag, breathing out as deeply as you can (making sure that you are not losing any air down your nose). Twist the top of the bag to keep the exhaled air in, and push the air sample down the bag towards the closed end.
- ▼ Read off the estimate of vital capacity from the scale.



Teaching NotesTwo

Two methods are given for this practical, since the equipment available may well vary in different schools/colleges.

The container method is simple, but since most labs will only have one or two large sinks, it is a slow process if you want the whole class to try it.

The plastic bag can be obtained from Biological suppliers, and the method is simple.

Requirements per student group - Method A

Simple container

Large sink

Screw cap or piece of plastic sheet to cover the opening of the simple container

Length of rubber tubing with plastic mouthpiece

Beaker of antiseptic

Marker pen

500 cm³ measuring cylinder or beaker

Requirements per student group - Method B

Long plastic bag with scale

Length of plastic tubing and disposable mouthpieces

Elastic band

Beaker of antiseptic

Traces from 'Box Type' Respirometer

Figure: Normal Tidal Breathing trace. Tidal volume and ventilation rate can be determined. If a static exercise can be arranged, e.g. exercise cycle the effect of physical exercise on these measures can be recorded.

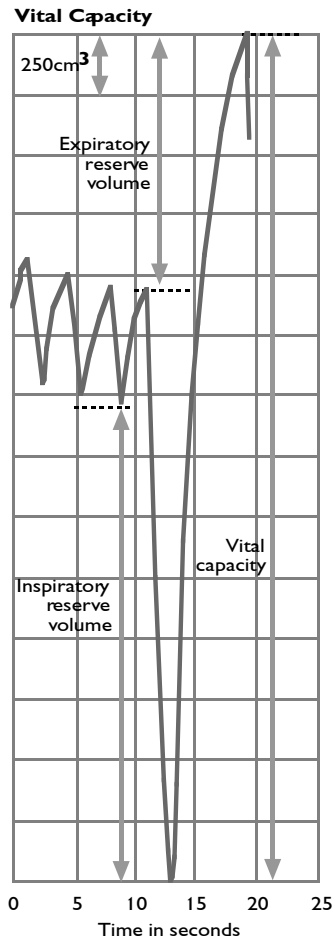
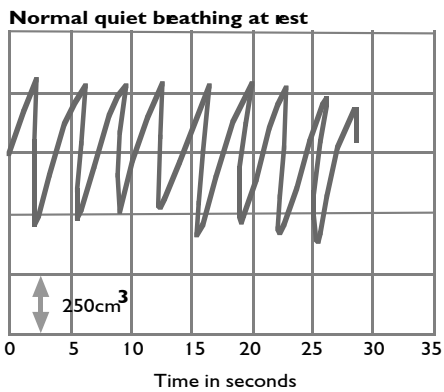
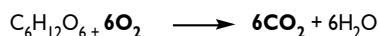


Figure: Vital Capacity Trace. The **vital capacity** is determined by taking a maximum inspiration then exhaling as hard and completely as possible, this can also be referred to as the Forced Vital Capacity (FVC).

Quantitative comparisons of inspired and expired air

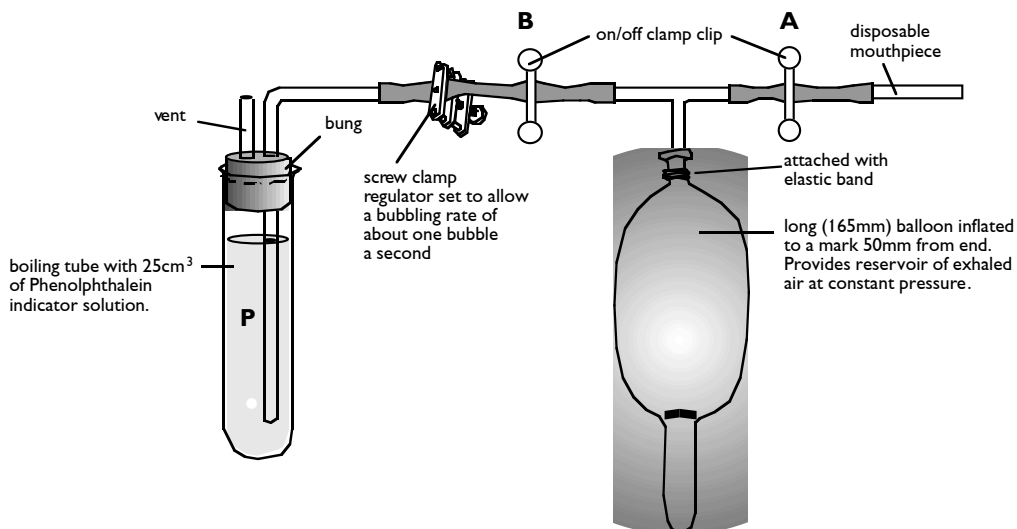
Introduction

In the simplest investigation using the 'Balloon respirometer' (Method A) the composition of inspired air can be taken as known (being the same as the composition of the air), and the amount of oxygen consumed at rest can be considered to be equal to the amount of carbon dioxide produced:



The 'J-tube Method' (Method B) analyses the oxygen and carbon dioxide content of both inspired and expired air, and the process is more complex.

Method Figure: Simple apparatus to determine a measure of the CO_2 content of air



- ▼ Close tap B and open tap A.
- ▼ Inflate the balloon with a small pump attached to the place otherwise occupied by the removable mouthpiece to the mark and close tap A.
- ▼ Open tap B and allow air to bubble through the alkaline indicator solution (*P*). CO₂ being an 'acid' gas will neutralise the solution *P*. Count the bubbles and record the time for the pink colour to disappear. There is only 0.04% CO₂ in air so it is unlikely that the pink colour will disappear. You will have to experiment with dilutions of the alkaline indicator solution and repeat balloon-fulls to achieve a result.
- ▼ Close tap B and open tap A.
- ▼ Inflate the balloon in one breath up to the mark and close tap A.
- ▼ Open tap B and allow air to bubble through the alkaline indicator solution (*P*). CO₂ being an 'acid' gas will neutralise the solution *P*. Count the bubbles and record the time for the pink colour to disappear.
- ▼ As long as care is taken with the rate of bubbling, the time to decolourisation is a crude measure of the relative amounts of CO₂ in the samples.

The apparatus can be used to make simple comparative measures of CO₂ production before, during and after exercise. Samples can be taken at timed intervals throughout the investigation, without disrupting the procedure.

$$\text{The ratio: } \frac{\text{Time to decolour P at rest}}{\text{Time to decolour P after a period of exercise}} = \text{CO}_2 \text{ Index}$$

Plot CO₂ Index and pulse rate against time on the same graph.

Teaching Notes

Time needs to be taken to establish a constant bubbling rate.

This involves inflating the balloon to the same extent each time, and having the screw clip adjusted correctly.

Requirements

Boiling tube with 25cm³ of phenolphthalein indicator solution

500 cm³ of phenolphthalein indicator solution prepared by dissolving 1g of solid phenolphthalein in 500 cm³ of distilled water and adding sodium hydroxide drop by drop until a pink colour is obtained.

Long balloon with a mark to establish a standard volume and pressure of inflation.

The apparatus as illustrated

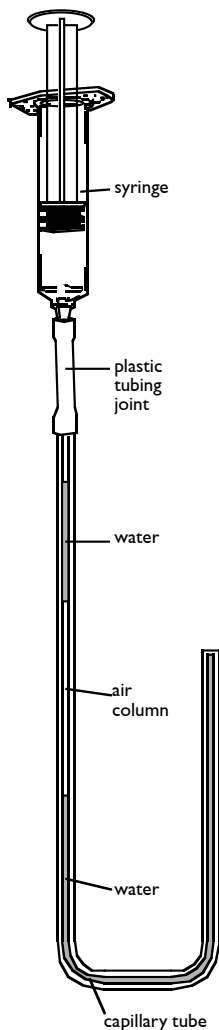
Disposable mouthpieces

Quantitative comparisons of inspired and expired air using a 'J' tube

In order to compare samples of air, it is necessary to analyse the level of CO_2 and O_2 contained in them. The simplest way to do this is to use a J-tube, which is a hook-shaped length of capillary tubing.

Air is drawn up into the tube, then solutions of potassium hydroxide and potassium pyrogallate are added to absorb the CO_2 and O_2 from the sample.

'J' tube for gas analysis



By measuring the amount of sample left it is possible to work out how much CO_2 and O_2 were in the original sample.

In this method, potassium hydroxide solution is used to absorb the CO_2 , then pyrogallol is added to the KOH. This reacts to make potassium pyrogallate within the J-tube, which absorbs the O_2 .

As the procedure requires some practice, and the solutions are toxic and corrosive, this experiment is best performed as a demonstration by the Supervisor.

continued

Teaching Notes

Quantitative comparisons of inspired and expired air using a 'J-tube'

This practical is fiddly, but it works quite well.

The most difficult aspect is getting used to the tiny amounts of pressure needed to move the air column: it is suggested you spend a couple of minutes working with the air column and water seals, before starting to use the more toxic substances.

The air column should be between 100 and 200 mm long. Much shorter columns do not give very measurable changes in length, and longer ones can be difficult to handle. When moving the air column back and forth, it should not be allowed to get up as far as the air / water space inside the syringe.

An outline method is set out below, but should be practised a few times and modified if necessary in the light of your experience.

- ▼ Label 4 small beakers a, b, c, and d. Wearing gloves and safety glasses, fill the beakers from one quarter to half full with the following materials:
 - a) Water
 - b) Potassium hydroxide (KOH - for absorbing carbon dioxide)
 - c) Pyrogallol (for absorbing oxygen)
 - d) Dilute hydrochloric acid (for cleaning the J-tube)
- ▼ Push the plunger of the syringe fully into the barrel. Draw a small quantity of water into the J-tube (about 5 cm long), then draw up approximately 10 cm length of air, then draw up some more water. This creates a sample of normal atmospheric air that can be analysed. Practice pushing the air column back and forth for a couple of minutes, until you have got the hang of the amount of pressure needed, the syringe movement will be tiny!
- ▼ Wait for at least 1 minute, and do not handle the capillary tubing where the air column is positioned. Measure the length of the column of air in millimetres and note it down (length A).
- ▼ Expel all but about 1 cm of the water from the open end of the J-tube, keeping this 1 cm to maintain a seal for the air column. Place the end of the tube in the potassium hydroxide solution and draw up a quantity of the KOH. Keeping the end of the tube in the KOH, push the air column back and forth in the J-tube, by using the syringe plunger, about 6 times, allowing the air column to come into contact with the sides of the tube wetted with the KOH solution. This will absorb the CO₂ from the air column. Remembering not to handle the capillary tubing, wait one minute, then re-measure the length of the air column and note down the new length (length B).
- ▼ Now expel all but 5 cm of the KOH from the J-tube. Place the end in the pyrogallol solution and draw up a sample. Push the air column back and forth in the J-tube as you did with the KOH, but DON'T expel the hydroxide. The KOH and the pyrogallol will react in the tube to form potassium pyrogallate, which will then absorb the O₂. Wait for 1 minute, then measure the length of the air column again (length C).

continued

- ▼ Expel all the contents of the J-tube into the sink and wash them away with water. Then wash the J-tube out thoroughly, first with water, then with dilute HCl, then with water again.
- ▼ Place a large test-tube in a bowl of water. Allow it to fill up with water, then raise it up to a vertical position, with the open end down, so it remains full of water. (See Diagram)
- ▼ Bend a clean bendy-straw, and place the bent end into the bowl of water and exhale into the straw from the straight end. As you get close to the end of your breath, place the test-tube over the bent end of the straw, and collect a sample of your exhaled air in the test-tube. It is best to collect a sample of air from as deep in your lungs as possible, so you should wait until you are almost out of breath.
- ▼ Keep the test-tube in the water to hold the sample of air in the tube. Draw up about 5 cm of water into the J-tube, then insert the end of the J-tube into the test-tube and move it up until the end is in your air sample. Draw up about 10 cm of your expired air sample, then about 5 cm of water to seal the sample.
- ▼ Test the sample of air using the KOH and pyrogallol in the same way you did before, and note the lengths of the column at each stage.
- ▼ If you have time, carry out some vigorous exercise for 3-5 minutes (for example run on the spot) then immediately, before you get your breath back, produce another sample of exhaled air in the same way you did in steps 6-8. Repeat the analysis and see if there are any differences.
- ▼ Calculate the percentages of oxygen and carbon dioxide in the air samples you have tested.

Requirements

J-tube apparatus, using 5 cm³ syringe if possible
4 x 50 cm³ beakers
Disposable bendy-straw
Boiling tube
Bowl, large enough to hold the boiling tube held horizontally
Safety glasses
Disposable gloves
Ruler marked in millimetres

Concentrated potassium hydroxide solution
Concentrated pyrogallous acid (pyrogallol)
Dilute hydrochloric acid
Tap water

Dissection of the Mammalian Heart

Method

You will be provided with a fresh or preserved sheep's heart. Examine the outer surface of the heart and identify the main blood vessels, and the position of the four chambers of the heart.

- ▼ Make sure you are looking at the ventral surface of the heart. Trace the position of the coronary artery and vein on the surface of the ventricles, starting at the opening of the artery from the aorta, and the entry of the vein into the right atrium respectively.
- ▼ Carefully cut open the two ventricles.
- ▼ Examine the interior of the right ventricle, and identify the tricuspid valve between the right atrium and the right ventricle. How many flaps does it have? Also examine the tendonous cords which are attached to the edges of the tricuspid valve. What tissues do you think they are made of, and what is their function? Look at the column-shaped papillary muscles, to which the cords are also attached. Note any congealed blood that may be present.
- ▼ Look for the semi-lunar valve leading to the pulmonary artery. Why do you think it is called a semi-lunar valve?
- ▼ Now examine the left ventricle. How does the bicuspid valve differ from the tricuspid valve? What difference can you see between the walls of the right and left ventricle, and why is this important?
- ▼ Attach a piece of rubber tubing to a water tap, and use it to run water into the blood vessels at the top of the heart (if they are present). By checking which ventricle the water enters, identify which are the venae cavae, and which are the pulmonary veins.
- ▼ Now turn the heart over and run water into the top of the aorta: what happens to the semi-lunar pocket valve in the aorta? Repeat the process with the pulmonary artery. How do these two pocket valves work?
- ▼ Run water into the right ventricle through the slit you have opened up. What happens to the tricuspid valve?
- ▼ Carefully cut into the right atrium and examine the tricuspid valve again. Cut into the left atrium and examine the openings of the pulmonary veins. Do they have valves in them?
- ▼ Now cut down the aorta from the open end and spread it out slightly. Examine the semi-lunar pocket valves from the other side. Also look for the opening of the coronary artery just above the semi-lunar valve.
- ▼ Compare the thickness and elasticity of the aorta and pulmonary artery with that of the pulmonary veins and venae cavae (if enough of these vessels remain). How is any difference in structure related to the functions of the two main types of blood vessel?

Teaching Notes

Preserved sheep's hearts can be obtained from biological suppliers. If you prefer to work with fresh materials, they can be obtained from a local abattoir or butcher, although the atria and major blood vessels and even the ventricles may be damaged. Pig's hearts may be used as an alternative.

Requirements per student

- Dissecting instruments and dissecting tray
- Disposable gloves
- Rubber tubing
- Access to a tap
- Sheep's heart

Water Loss in Plants

Loss of mass from leaves under different conditions

Introduction

The loss of mass from leaves will be measured. It is assumed that any loss of mass that occurs is caused by transpiration, i.e. evaporation of water through the stomata of the leaf. A count of the number of stomata on the two surfaces of the leaf will be taken and related to the rate of transpiration.

Method

- ▼ Collect 4 large leaves, with the leaf stalk attached, from the plant material provided. Find some way of identifying which leaf is which, e.g. by tying coloured thread around the stalk of each leaf, leaving long ends which you can use later. Do not write on the leaf surface with pens or markers.
- ▼ Dip the ends of all the leaf stalks in Vaseline to seal them. This prevents water loss from the cut ends.
- ▼ Each group should tie a length of string between two stands or two taps, so that the leaves can be suspended in the air, to allow free movement of air around them.
- ▼ Treat the four leaves as indicated below:
 - A: Leave untreated as a control;
 - B Smear the whole lower surface of the leaf with Vaseline: do not get any on the upper surface;
 - C Smear the upper surface of the leaf, in a similar way;
 - D Smear both surfaces.
- ▼ Tie the cotton ends of each leaf to a paperclip, and bend the wire end of the paperclip out slightly so it can be used to hook the leaf over the string support. Weigh each leaf, once it has been treated and the clip attached, and note the mass of the treated leaf (cotton, clip and all!).
- ▼ Hook each leaf over the string using the paperclip, so they are separated and air can flow freely around them. Try to ensure there is no draught around the leaves. Start your stopclock.

continued

- ▼ At 20-minute intervals, re-weigh each leaf in turn and note the new mass. When you are moving the leaves, be careful not to touch them if you can avoid it. Handle the paperclip only. Continue to weigh the leaves every 20 minutes for 1 - 2 hours.
- ▼ Record the results in a suitable table and calculate the percentage loss of mass for each leaf (remember to remove the weight of the paper clip from your calculations). Consider how the different treatments have affected the rate of loss of mass.
- ▼ Repeat the experiment, using fresh leaves, and putting the leaves in a draught of warm or cool air, e.g. from a hairdryer or fan heater.
- ▼ While your experiment is continuing, collect two fresh leaves (the same type as before), and paint a small amount of clear nail varnish on the upper surface of one leaf, and the lower surface of the other (about 1cm square in each case). Do not forget to continue your measurements of mass loss from the other leaves!
- ▼ Allow the nail varnish to dry thoroughly, then carefully peel the nail varnish layer off the lower surface, using forceps. Place the layer of nail varnish on a microscope slide in a drop of methylene blue, and make sure it is lying flat. Place a coverslip over the nail varnish layer. The nail varnish makes an imprint of the cell layer on the surface of the leaf, so you can see the surface layer without having to peel the epidermis itself off the leaf.
- ▼ Examine the lower surface imprint using the medium power ($\times 10$) objective lens of the microscope. Identify the stomata and guard cells on the leaf surface. Count how many stomata you can see in the field of view of the microscope. Then move the microscope to another area of the layer and count how many stomata are visible.
- ▼ Repeat the count five times, and calculate a mean number of stomata per field of view.
- ▼ Repeat the process with the leaf upper surface nail varnish layer, and calculate the mean number of stomata per field of view on the upper surface. How do these figures relate to the results of your leaf mass experiment?

- ▼ Record the results in a suitable table and calculate the percentage loss of mass for each leaf (remember to remove the weight of the paper clip from the calculations). Consider how the different treatments have affected the rate of loss of mass.
- ▼ Repeat the experiment, using fresh leaves, and putting the leaves in a draught of warm or cool air, e.g. from a hairdryer or fan.
- ▼ While the experiment is continuing, collect two fresh leaves (the same type as before), and paint a small amount of clear nail varnish on the upper surface of one leaf, and the lower surface of the other (about 1cm square in each case). Do not forget to continue the measurements of mass loss from the other leaves!
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- ▼ Repeat the count five times, and calculate a mean number of stomata per field of view.
- ▼ Repeat the process with the leaf upper surface nail varnish layer, and calculate the mean number of stomata per field of view on the upper surface. How do these figures relate to the results of the leaf mass experiment?

Teaching Notes

The standard way of examining transpiration is to use the potometer. However, this is difficult to set up and get to work consistently. This alternative works well and is much easier to carry out, although the criticism could be levelled against it that water loss from a detached leaf will be very different from one with a water supply.

Provided the plant material is freshly cut, or ideally provided as a pot plant, this experiment works well and is simple to set up.

If you have enough plant material, it would be best for each group to use 3-4 leaves for each treatment. This would give a larger and more measurable mass loss, and more valid results.

If you are short on time, some groups within the class can work without air draughts, others with cool or warm draughts, and then they could combine their results.

It is suggested that you should set up a bubble potometer experiment for the students to see as a demonstration, so they can understand questions based on its use. Combined with the understanding of factors which affect transpiration gained from the leaf greasing experiment, this should allow them to answer any questions based on the potometer.

Requirements per student

- Length of string, about 1-2 m
- Lengths of cotton thread in 4 different colours
- Vaseline
- Paper clips
- Clear nail varnish
- Forceps
- Microscope
- Slide and coverslip
- Methylene blue in a dropping bottle
- Balance
- Hairdryer or fan heater
- 2 clamp stands
- Stopclock
- Supply of freshly cut plant material, from a plant with large leaves.
(Choose a non-hairy variety of plant)

The observation of xylem vessels, sieve tube elements and companion cells using the light microscope

Introduction

The xylem elements are clearly seen in both L.S. and T.S. as a result of their size and thick lignified walls (usually stained red), but the phloem poses problems in some specimens. Often the only clear guide to the identification of phloem in T.S., apart from its location, is the appearance of sieve tube plates, which are only occasionally caught in T.S. In L.S. sieve plates will always be seen. The companion cells are more difficult to see in both planes of section.

Method

- ▼ Collect prepared slides of a longitudinal section and a transverse section through a plant stem. Examine the slides under the low power (x4) objective lens of the microscope.
- ▼ Locate the xylem and phloem tissues, and adjust the objective lenses of the microscope so you can see details of the cell structure.
- ▼ Identify the large red stained xylem vessels, and the smaller, thinner-walled phloem elements. You may be able to see the companion cell next to each phloem sieve tube. Draw examples of the three types of cell: xylem vessels, phloem sieve tubes, and companion cells, as seen in both LS and TS. Label, annotate and indicate the final magnification of the drawings. Include suitable headings.
- ▼ Draw and complete a suitable table to compare the three types of cell, with regard to their structure and function(s)

Teaching Notes

This practical is fairly straightforward, although students may need to have diagrams of the structure of a dicotyledonous plant stem, and of the individual cells, to help with identification. The xylem vessels are usually the largest elements in the vascular bundle, with no cytoplasm and lignin stained red. The phloem elements are smaller and thinner-walled: if the sieve plates are visible in the TS they are easier to identify. The companion cells are always adjacent to the phloem sieve tube elements, but it may not be easy to identify them definitively, especially in the L.S.

The most useful part of this practical is probably the discussion afterwards concerning differences in structure between the cell types, and how these relate to the functions of the cells within the whole plant.

This practical also provides an opportunity to measure accurately using the eye piece graticule, e.g. the diameter of xylem vessels, and sieve plates.

Requirements per student:

Prepared slides of LS and TS of plant stem, e.g. *Cucurbita* and *Helianthus*
Microscope
Drawing materials.
Reference books/photos etc.

Demonstrations and measurements of transpiration using a potometer

Introduction

Transpiration can be measured using a potometer. The principle of which, is that as the water evaporates from the leaves of a shoot, water is pulled up the shoot by the cohesive pull of the transpiration stream, and an air bubble is drawn along a capillary tube at the same rate - thus giving a measure of the rate of transpiration.

The time taken for the air bubble to travel a certain distance, or the distance travelled in a certain time, can be recorded and the rate of transpiration calculated.

If placed on a sensitive electronic balance, the loss of mass represents the loss of water by transpiration from the leaves.

If a set of consistent results is obtained, the figures of the uptake and of the loss of water, can be compared.

Method

- ▼ The potometer is filled by immersing it totally in water, preferably freshly boiled and cooled water which has no air bubbles.
- ▼ The shoot to be used should be cut from the plant under water, to prevent any air bubbles entering the xylem elements, and kept under water until it is inserted into the apparatus
- ▼ All joints in the apparatus should be well vaselined to prevent any leaks, which would invalidate the results by reducing the apparent rate of transpiration.
- ▼ After setting up, but before allowing air to be drawn into the capillary tube, the apparatus should be allowed to come into equilibrium, with the end of the capillary tube being kept under water until such time as an air bubble is introduced.
- ▼ An air bubble is introduced by lifting the capillary tube out of the water to allow air to be drawn up, and then returning the tube to the water.
- ▼ The time it takes for the bubble to travel a set distance along the tube is then recorded.
- ▼ After the bubble has travelled a certain distance, it is returned to the beaker end of the tube by running water in from the reservoir.
- ▼ The procedure is repeated until some consistent results are obtained.

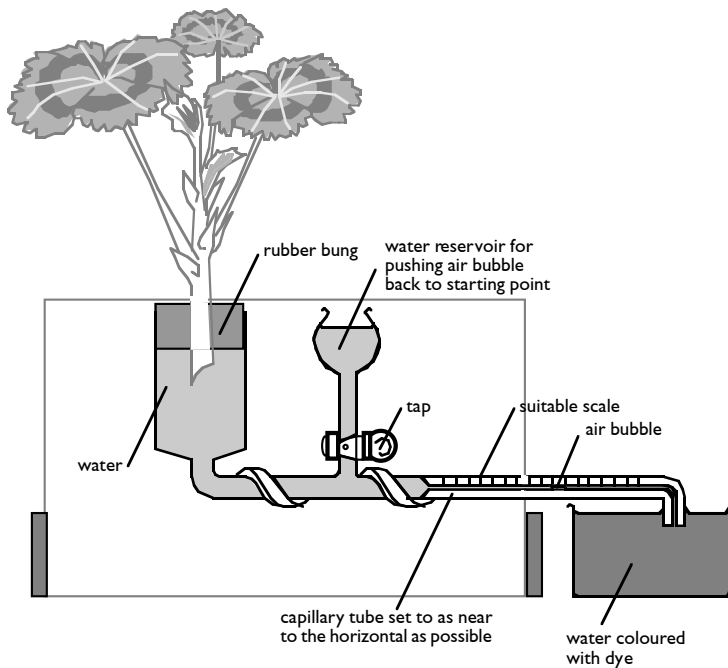
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- ▼ If the calibration on the capillary tube is in millimetres, and the diameter and hence the radius of the tube is known, then the volume of water taken up can be calculated using the formula:

$$\pi r^2 \times \text{distance travelled by bubble} = \text{volume of water uptake}$$

Sometimes, however, the calibrations are already in units of volume, for example mm^3 or cm^3 (ml).

Express the results as either cm^3 per minute, cm^3 per hour, or cm^3 per hour per cm^2 of leaf area.



Teaching Notes

The experiment is often run without a bubble, just with the end meniscus as the point of measurement. Strictly speaking this is less valid than using a bubble, as it is possible for water to evaporate from the open meniscus, thus increasing the apparent rate of water uptake - although in reality the effect is immeasurable.

The potometer actually measures the rate of water uptake, which under these experimental conditions is not the same as the transpiration rate. In this experiment the cross sectional area of the cut shoot is not as large as the equivalent area of root hairs that would normally supply the leaves, therefore the rate of uptake is less than the rate of transpiration.

Lengthy attempts to set up a whole plant complete with its root system in a potometer, in order to increase the rate of movement of the meniscus, and overcome the problem of the cut stem not providing enough surface area for water uptake, all failed for unknown reasons.

If set up successfully the apparatus can be used to compare rates of transpiration in different environmental conditions. e.g. still air and moving air (possibly using a hair dryer or fan).

The apparatus could also be set up with different leaf surfaces greased.

Given ideal results, it would be found that the loss of water as determined by placing the apparatus on an electronic balance for the duration of the experiment, is greater than the amount of water uptake as determined by the movement of the bubble.

Requirements per group

Potometer set up and ready to go

or

Potometer

Vaseline

100 cm³ beaker, water and dye

Large sink

Suitable leafy shoot cut in the correct way, and to fit the apparatus

Hair dryer or fan if required